Environmental impact of mine drainage and its treatment on aquatic communities

by

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ABSTRACT

An ecological and chemical analysis of eight Welsh streams impacted by mine drainage is used to discern the effects of water and sediment related variables in the impact of mine pollution on freshwater macroinvertebrate communities. The implications of this are to be considered for improving mine water remediation techniques and work towards the achievement of the environmental objectives set by the EU Water Framework Directive (WFD).

Streams impacted by coal and metal mine drainage present a clear ecological impact in response to water and sediment related variables, demonstrating that both sediment and water are key aspects in mine drainage pollution of freshwater ecosystems. However, the WFD does not include metal concentration guidelines for sediments, neither has the UK set mandatory standards for them, and sediments are not currently being routinely monitored or remediated in the UK.

To achieve the environmental objectives set by the WFD, the Coal Authority and the Environment Agency are constructing several engineered wetlands in the UK to treat mine drainage. One of these constructed engineered wetlands was seen to successfully remediate mine water removing trace metals and suspended solids and increasing pH and dissolved oxygen. However, the remediation scheme seemed to fail to improve the electrolyte status of the water and stream sediment quality. As a result, the benthic community in the receiving stream appeared to have a poor recovery.

Als meus pares
(To my parents)

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I am indebted to the support given to me by my family, not just throughout the duration of this Ph.D, but throughout the whole of my academic career. My thanks go also to my nieces and nephew for helping me maintain prospective on life. Many thanks to Chris and Mike for making me feel at home in the edge of the Yorkshire Wolds. I am also deeply indebted to Peter for his unconditional support and encouragement.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF ABREVIATIONS

AAS: Atomic Absorption Spectrophotometry

AFDM: Ash free dry mass

CA: The Coal Authority

CCA: Canonical Correspondence Analysis

CPOM: Coarse organic particulate matter

DCCA: Detrended canonical correspondence analysis

DO: Dissolved oxygen

EA: Environment Agency

EC: Electrical conductivity

EPT(C): Ephemeroptera, Plecoptera and Trichoptera (and Chironomida)

EQS: Environmental Quality standards

FPOM: Fine organic particulate matter

GAM: Generalised Additive Modelling

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

PEL: Predicted Effect Level

RBPM: River Basin Management Plans

RDA: Redundancy Analysis

SEPA: Scottish Environment Protection Agency

SS: suspended solids

TDS: Total dissolved solids

TEL: Threshold Effect Level

UKTAG: UK Technical Advisory Group on the Water Framework Directive

WFD: Water Framework Directive

1 INTRODUCTION

1.1 Introduction

Mine pollution represents a serious environmental problem in countries which have a mining history such as the UK. Mining extractions in the UK pre-date Roman occupation (Keen, 1996), and most mines were abandoned at the end of the 19th century and beginning of the 20th century (Keen, 1996, Bick, 1974). Drainage from these abandoned mines into surface and ground water bodies causes 9% of rivers in England and Wales and 2% in Scotland to be at risk of failing to meet the objective of 'good ecological' status set by the EU Water Framework Directive (Johnston and Rolley, 2008).

During mining extractions, ground water levels are kept below the depth of the works, but once the mine is abandoned, galleries are flooded as the water table re-bounds to its original level. This water gets in touch with the atmosphere in the galleries and its dissolved oxygen concentration increases. The minerals exposed in the walls of the galleries react with the oxygenated water and release metals. Pyrite (FeS_2) is typically the main mineral found in coal mines, and when oxidised produces acidity, iron and sulphate. Iron reacts further in the oxygenated water to produce orange coloured ochre deposits (iron oxyhydroxides), typically observed in streams polluted by coal mine drainage. In non-ferrous metal mines (hereafter referred to as 'metal mines'), mineral oxidation also releases trace metals, such as zinc and lead, and sulphates, although acidity is not

necessarily produced (Younger, 2002). In these mines, the metals released depend on the mineral found in the bedrock.

Both coal and metal mine drainage has been seen to impact the ecosystems in receiving water bodies (Koryak et al., 1972, Tomkiewicz and Dunson, 1977, Jarvis and Younger, 1997, Gray, 1998, Hickey and Clements, 1998, Kelly, 1999, Malmqvist and Hoffsten, 1999, Richardson and Kiffney, 2000, Peeters et al., 2001, DeNicola and Stapleton, 2002, Van Damme et al., 2008). However the relationship between environmental variables and the response of the ecosystem is poorly understood. In particular the relative importance of sediment and water chemistry in controlling ecosystem response needs to be established. A better understanding of the processes occurring in these streams can help optimise remediation techniques needed to meet the requirements established by the EU Water Framework Directive.

1.2 The European Union Water Framework Directive

The Water Framework Directive 2000/60/EC (WFD) is a piece of legislation from the European Union (EU) that came into law in December 2000. The directive seeks the protection of inland surface water, ground water, transitional (estuarine) water and coastal water from member states, and its specific Environmental Objectives are as follows (UKTAG, 2008):

1. Prevent deterioration of the status of all surface and ground water bodies;

2. Protect, enhance and restore all bodies of surface and ground water with the aim of achieving good ecological status by 2015, the first of the 3 six year planning cycles: 2015, 2021, 2027.

The WFD defines two types of chemical substances that need to be monitored, and thresholds above which damage to the aquatic ecosystem may be incurred (Environmental Quality Standards or EQS). *Priority Hazardous Substances*, are those chemicals with higher potential impact on aquatic ecosystems, and include hydrocarbons, pesticides and some metals (cadmium and mercury, with the possibility to add lead and nickel in the near future) (UKTAG, 2008). EQS for these substances are defined by the European Parliament and the Council of Ministers and are applicable to all EU member states. *Specific pollutants* are substances that can be harmful to ecological communities and may be identified by member states as being discharged to water bodies in 'significant quantities', including cyanides, arsenic and other metals (UKTAG, 2008). EQS for these substances may be defined by each member state. In the case of Great Britain, the UK Technical Advisory Group on the WFD (UKTAG) is the responsible body. Table 1.1 displays some EQS derived from the WFD published in the directive programme by the Environment Agency (EA) that are applicable to the study of streams polluted by mine drainage.

The implementation of the WFD is administrated through River Basin Management Plans (RBMP), which describe the current condition of each river basin and identify those at

risk of failing the environmental objectives. Water status is classified at 5 levels (high, good, moderate, poor and bad) considering chemical, ecological and morphological aspects of the river basin. The ecology is measured by 'biological quality elements', which include phytoplankton, macrophytes, phytobenthos, benthic invertebrates and fish. When water bodies fail the target of 'good ecological status', member states are required to establish a programme with the actions needed to deliver environmental improvements by 2015, or the next six year planning cycles (2021, 2027) as long as there is no further deterioration.

Table 1.1 Environmental Quality Standards (EQS)

Substance	EQS (ppm)
Nitrate	17.5
Sulphate	87.5
Phosphate	0.04*- 0.1[+]
Zinc	0.12
Iron	1
Manganese	0.0175
Cadmium	0.001
Copper	0.001
Lead	0.004
Chromium	0.005
Nickel	0.05
pH	6-9

* calcareous rivers
[+] non-calcareous rivers

Current work undertaken in the UK by the EA and the Scottish Environment Protection Agency (SEPA) have identified major causes of failure to meet 'good ecological status' in British surface and ground water bodies. These include: flow regulations for electricity generation, diffuse pollution from agriculture and urban land, point source pollution from sewage, changes in the morphology of water bodies from engineering activities and

agriculture, quarrying and mining (EA, 2008, SEPA, 2009). Eight of the twelve River Basin Districts defined for the RBMP in the UK identify mine pollution as a significant problem, resulting in 9% of rivers in England and Wales and 2% in Scotland being at risk of failing to meet good ecological status due to mine pollution (Johnston and Rolley, 2008).

In order to tackle coal mine pollution problems within the framework of the WFD in the UK, The Coal Authority (CA) has developed a program of remedial and preventive measures, currently consisting of forty six treatment schemes cleaning and protecting 200km of rivers (Johnston and Rolley, 2008). Future remediation schemes are planned to be constructed within the next three planning cycles established by the WFD according to their risk: high (by 2015), medium (by 2021) and low (by 2027).

In the case of metal mines, there is no national body responsible for their pollution control and remediation. However, the EA and SEPA have included metal mine monitoring and remediation as part of their program for protecting the environment. The EA has identified 315 surface water bodies in England and Wales at risk or probably at risk of failing to achieve the WFD environmental objectives due to metal mine drainage pollution (Johnston and Rolley, 2008). The EA Wales has defined a Metal Mine Strategy, identifying the top 50 metal mines causing the greatest environmental risk, which are priority for treatment (Mullinger, 2004). All the metal mines studied in the present project are found within this top 50. However, metal mine drainage remediation is technically more complicated than coal mine drainage, hence only one full-scale treatment scheme has been built in the UK so far: the Wheal Jane tin mine in Cornwall (Johnston and

Rolley, 2008). Nevertheless, there is ongoing study in the UK for metal mine remediation with pilot-scale plants in Wales, Cornwall and North Pennines (Johnston and Rolley, 2008), and a major EU funded project is being designed by the EA, the CA and the Welsh Assembly Government to remediate Cwm Rheidol mine drainage and improve the ecological quality of Afon Rheidol (Edwards and Potter, 2007).

1.3 The role of sediment in mine pollution and the WFD

Mine drainage often increases aqueous metal concentrations in receiving water bodies, and these can be transferred to sediments by adsorption and precipitation mechanisms (Smith, 1999). Additionally, erosion, transport and deposition of contaminated sediments can be an important source of trace metals many kilometres downstream from the mine input (Hudson-Edwards et al., 2008). Sediment-bound metals are thought to represent as much as 90% of those derived from mine drainage (Hudson-Edwards et al., 2008), and, in similar fashion to aqueous species, are thought to cause ecological damage on receiving water bodies, resulting in failure to achieve good ecological status in many British river basins. However, the WFD does not include sediment metal concentration guidelines, neither has the UK set mandatory standards for them. As a consequence, no British rivers are currently being remediated to improve poor sediment quality (Hudson-Edwards et al., 2008), and sediment quality measurements are rare in routine monitoring.

Benthic organisms are thought to take up metals while burrowing in contaminated sediment or when feeding from particulate matter (Bervoets et al., 1997, Bervoets et al., 1998), although this has not always been proved (Van Damme et al. 2008). Additionally,

there are many factors determining bioavailability and toxicity of sediment-bound metals (e.g. pH, organic matter) and exposure routes between sediment and organisms are not fully understood (Hudson-Edwards et al., 2008). Thus, establishing sediment metal concentration guidelines is a complicated task.

The EA has initiated a programme to establish sediment quality guidelines in relation to metal contamination and mining legacy in the UK, based on the Environment Canada 'Threshold Effect Level' (TEL) and 'Predicted Effect Level' (PEL) (Hudson-Edwards et al. 2008). TEL is the metal concentration in sediments below which there is no associated ecological impact, whereas PEL is the lower concentration at which ecological damage has been observed. There is still ongoing research to determine such guidelines, but an initial EA draft suggests TEL and PEL for some metals: arsenic, cadmium, copper, lead and zinc (Hudson-Edwards et al. 2008) (Table 1.2). The same report, compares TEL and PEL with metal concentrations in sediments from several British river basins known to be impacted by metal mine pollution, observing that many fail these proposed guidelines for one or more metals. Examples include sediments from the River Swale (Yorkshire) (up to 22mg/Kg Cd, 4818 mg/Kg Pb and 12203 mg/Kg Zn) (Macklin et al. 1994) and Afon Rheidol floodplain (up to 4.4 mg/Kg Cd, 76mg/Kg Cu, 2520 mg/Kg Pb and 680 mg/kg Zn) (Swain et al. 2005).

Table 1.2 Threshold Effect Level (TEL) and Predicted Effect Level (PEL) for arsenic (As), cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn). From Hudson-Edwards et al. (2008).

	As (mg/Kg)	Cd (mg/Kg)	Cu (mg/Kg)	Pb (mg/Kg)	Zn (mg/Kg)
TEL	5.9	0.596	36.7	35	12.3
PEL	17	3.53	197	91.3	315

1.4 Aims and objectives

To achieve the environmental objectives set by the WFD, it is crucial to understand how mine pollution affects freshwater ecological communities, working towards optimisation of remediation and prevention techniques. The present study investigates the relationship between environmental variables related to mine pollution and the ecology in eight Welsh streams, with a focus on discerning the effects of water and sediment bound contaminants. In addition, a stream in Wigan (Manchester) is monitored before and after the CA constructed an engineered wetland to treat drainage from an abandoned colliery. This study provides a unique opportunity to test the efficacy of remediation techniques and to determine if water treatment alone is enough to induce ecological recovery of streams polluted by coal mine drainage. Finally, a stream polluted by metal mine drainage (Afon Rheidol) is intensively studied to identify the major impacts of Cwm Rheidol mine and characterise its ecological quality. This information will be used by the EA to optimise remediation techniques that will be installed in the site as part of an EU funded project.

The main objective of the present study is to identify the relationship between environmental variables linked to mine drainage pollution and macroinvertebrate communities, in order to apply the knowledge to mine water remediation. Specific

objectives tackled in different chapters of the present thesis are displayed in Figure 1.1 and include:

1. Determine the impact of coal and metal mine drainage on stream water, sediment and ecology;

    a. Determine the environmental variables (water and sediments) affected by mine drainage pollution which will be used within the ecological study (Chapter 3, Sections 3.4.1.1 and 3.4.2.1);

    b. Establish whether metals are predominantly found in the water or sediment phase within different impacted streams (Chapter 3, Sections 3.4.1.2 and 3.4.2.1);

    c. Identify the key differences in water chemistry between coal and metal mine drainage (Chapter 3, Section 3.5);

    d. Determine if abandoned mine drainage impacts on the structure of the aquatic community in receiving streams (Chapter 4, Sections 4.5.1 and 4.5.2);

    e. Determine if abandoned mine drainage negatively affects the function of the aquatic community in receiving streams (energy inputs and functional structure) (Chapter 5, Sections 5.5.1 and 5.5.2);

2. Determine the relationship between specific environmental variables derived from coal and metal mine drainage and aquatic communities;

    a. Assess the relative importance of water related variables and sediment related variables in predicting macroinvertebrate community structure at the sampled streams (Chapter 4, Sections 4.5.1 and 4.5.2);

    b. Relate environmental variables to changes in benthos composition (Chapter 4, Sections 4.5.1 and 4.5.2);

    c. Detect differences in benthos response to coal and metal mine drainage pollution (Chapter 4, Section 4.6);

    d. Relate environmental variables to functional changes in the benthic community (Chapter 5, Sections 5.5.1 and 5.5.2);

    e. Detect differences in ecosystem functional response to coal and metal mine drainage pollution (Chapter 5, Section 5.6);

3. Investigate the ecological and chemical response of a polluted stream to mine water remediation (Chapter 6);

    a. Establish if there is water and sediment quality recovery after the remediation scheme becomes operational (Section 6.5.1);

    b. Determine response times and spatial variations for the different environmental variables (water and sediment) related to mine pollution (Sections 6.5.2 and 6.5.3);

    c. Investigate if there is ecological recovery and establish a timescale (Section 6.5.4);

4. Study of a stream currently receiving metal mine drainage (Cwm Rheidol) prior to the design of a remediation scheme (Chapter 7);

    a. Determine if Cwm Rheidol mine is negatively impacting on Afon Rheidol water, sediment, and ecological quality (Sections 7.4.1 and 7.4.2);

    b. Determine if there is spatial recovery as the river flows downstream from the mine discharge (Section 7.4.1 and 7.4.2);

    c. Investigate differences in water chemistry, trace metal concentration in sediments and ecology between riffles and pools (Sections 7.4.1 and 7.4.2).

1.5 Thesis structure

Chapter 1 introduces the work presented in this thesis. A general background on the legislation that triggers most of the work currently undertaken on water pollution in Europe, and therefore the present study, has been presented. The specific literature review related to different aspects of mine pollution studied in this project can be found in the relevant chapters.

Chapter 2 provides a description of the sites sampled for the main part of the present study (Chapter 3-5) and general methodology. Methods only used in specific parts of the study and other sites are described in the relevant chapters.

Chapter 3 offers a chemical analysis of water and sediment from streams impacted by coal and metal mine pollution. This chapter identifies the main chemical variables affected by mine pollution, which are then used in the ecological part of this study.

Chapter 4 proceeds to investigate how the structure of macroinvertebrate assemblages is affected by mine pollution and how this relates to specific variables. Particular attention is focused on discerning the effect of water and sediment related variables.

Chapter 5 continues analysing the effects of mine pollution on stream function, expressed as algal growth, litter decomposition and assemblages of benthic functional feeding groups. The variation of functional feeding groups abundance and species richness is related to environmental variables.

Chapter 6 investigates the chemical and ecological recovery of a stream severely impacted by coal mine pollution after a remediation scheme is constructed by the CA. Data gathered in this study allow to determine if water remediation alone is enough to induce ecological recovery in a stream, and to evaluate the efficiency of engineered wetlands in coal mine drainage remediation.

Chapter 7 consists in a case study funded by the EA to provide preliminary data on the ecological status of Afon Rheidol. This will contribute to the design of a remediation scheme at the site funded by the EU. Results from this study also investigate differences between pools and riffles in terms of sediment chemistry and ecology, and the effect of spoil heap run off as a part of mine pollution.

Chapter 8 summarises the findings from the present study and their application into national programmes to achieve the environmental objectives established by the WFD in streams that have failed/are at risk of failure due to mine pollution. Additionally, it depicts questions aroused by this thesis that need further study.

(Next page) **Figure 1.1** Summary of main objectives and structure of the present thesis and linkage between them

IMPACT OF MINE DRAINAGE AND ITS TREATMENT ON AQUATIC COMMUNITIES

(1) Determine the impact of coal and metal mine drainage on stream water, sediment and ecology
(2) Determine the relationship between specific environmental variables derived from coal and metal mine drainage and aquatic communities
(3) Investigate the ecological and chemical response of a polluted stream to mine water remediation

Chemistry

(Chapter 3)

- Determine the environmental variables (water and sediments) affected by mine drainage pollution which will be used within the ecological study (Sections 3.4.1.1 and 3.4.2.1);
- Establish whether metals are predominantly found in the water or sediment phase within different impacted streams (Sections 3.4.1.2 and 3.4.2.1);
- Identify the key differences in water chemistry between coal and metal mine drainage (Section 3.5)

Ecology

(Chapters 4-5)

- Determine if abandoned mine drainage impacts on the structure of the aquatic community in receiving streams (Chapter 4, Sections 4.5.1 and 4.5.2);
- Detect differences in benthos response to coal and metal mine drainage pollution (Chapter 4, Section 4.6);
- Determine if abandoned mine drainage negatively affects the function of the aquatic community in receiving streams (energy inputs and functional structure) (Chapter 5, Sections 5.5.1 and 5.5.2);
- Detect differences in ecosystem functional response to coal and metal mine drainage pollution (Chapter 5, Section 5.6)
- Assess the relative importance of water related variables and sediment related variables in predicting macroinvertebrate community structure at the sampled streams (Chapter 4, Sections 4.5.1 and 4.5.2);
- Relate environmental variables to changes in benthos composition (Chapter 4, Sections 4.5.1 and 4.5.2);
- Sections Relate environmental variables to functional changes in the benthic community (Chapter 5, 5.5.1 and 5.5.2);

Recovery

(Chapter 6)

- Establish if there is water and sediment quality recovery after the remediation scheme becomes operational (Section 6.5.1);
- Determine response times and spatial variations for the different environmental variables (water and sediment) related to mine pollution (Sections 6.5.2 and 6.5.3);
- Investigate if there is ecological recovery and establish a timescale (Section 6.5.4

(4) Study of a stream currently receiving metal mine drainage (Cwm Rheidol) prior to the design of a remediation scheme (Chapter 7)

- Determine if Cwm Rheidol mine is negatively impacting on Afon Rheidol water, sediment, and ecological quality (Sections 7.4.1 and 7.4.2);
- Determine if there is spatial recovery as the river flows downstream from the mine discharge (Section 7.4.1 and 7.4.2);
- Investigate differences in water chemistry, trace metal concentration in sediments and ecology between riffles and pools (Sections 7.4.1 and 7.4.2).

2 METHODOLOGY

2.1 Site description

Eight Welsh streams, four impacted by metal mine drainage (Mid Wales) and four impacted by coal mine drainage (South West Wales), described below, form the field sites for Chapters 3-5 of this thesis. Additional field sites relating to Chapter 6 (Pemberton mine, Wigan) and Chapter 7 (Cwm Rheidol mine, Mid Wales) are described in the relevant chapters.

2.1.1 Climate at the study sites

British climate can be divided in four zones, and Wales belongs to the southwest area, experiencing mild winters, due to maritime influence, and warm summers. In terms of precipitation, Wales, as well as the whole of western Britain, is characterised by high rainfall. This is due to frontal depressions coming from the Mid-Atlantic, entering the country from the west and discharging as they reach altitude due to the Welsh mountains (Met Office, 2009).

Yearly rainfall average at Cardiff (South Wales) is 1111.7mm and average maximum and minimum temperatures are 14.3ºC and 6.8ºC respectively (1971-2000, Met Office). However, rainfall averages in the same station during the duration of this study were higher, being 1129.7mm in 2006 and 1290.8mm in 2007 (Met Office, 2009). In Trawscoed (Mid Wales) the yearly mean rainfall is 1213.9mm and average maximum and

minimum temperatures are 13.5ºC and 5.9ºC respectively (1971-2000, Met Office). Similarly in this area (Cwm Ystwyth station) the rainfall during the duration of this study was higher than the climatic average, being 1569.5mm in 2006 and 1798.4mm in 2007 (Mett Office, 2009).

2.1.2 Streams impacted by abandoned coal mines

Records of coal mining in South Wales date to before the 16th century, reaching a peak just before the First World War (Keen, 1996). Four abandoned coal mines from this area (Figure 2.1) were selected for study (Table 2.1). All the study sites were located on Palaeozoic sandstone, mudstone and shale, with clay peaty soils (Soil Survey of England and Wales, 1983). The four collieries were located in coal fields formed, as most of the coal in the UK, during the Carboniferous period. The coal of Cwm Gross and NantyFyllon mines came from the Upper Cwmgorse Marine band, whereas coal from Clyne and Aberbeiden mines originate in the Cefn Coed Marine band (BGS, 1997). Both coal bands were formed in marine or estuarine environments, and are therefore characterised by a higher proportion of iron sulphides and organically bound sulphur than coal formed in freshwater environments (Langmuir, 1997).

The four coal mines studied were underground works and had no remediation schemes associated with them. All the sampling sites were found below 200m above sea level, their location is displayed in Figures 2.1-2.5. Generally, up and downstream sampling points were only separated by a few meters, as one single outflow from the underground works was identified. Aberbeiden site (Figure 2.2) was the only exception, where

sampling points were separated by approximately 600m to include several mine outflows observed in the field. Table 2.1 summarises detailed information of each site.

The proximity between upstream and downstream sampling points only allows identifying local impacts within the streams, however downstream attenuation due to factors such as dilution from tributaries and natural buffer capacity of the streams (Cairns et al., 1971) is not considered here as this is out of the scope of this study.

Table 2.1 Description of the four study sites impacted by coal mine drainage

Mine (site's name)	Watercourse and Catchment	Grid reference	Date of closure[1]	Description
Aberbeiden	Craig yr Aber, Ogmore	SS8564 8482	1959	Situated in forestry commission land. First sampling point was upstream from the footbridge and the other sampling point was located 600m downstream, in order to include several underground outflows from the mine. Even though located close to Port Talbot, the surrounding area was not heavily urbanised
Cwm Gross	Nant Melyn, Tawe	SN7029 1095	1964	Surrounded by grassland occupied by horses and natural wetland. Very close to Cwm Gross village, by the industrial estate. Sampling points were established about 2m upstream and downstream from the mine discharge
NantyFyllon	NantyFyllon, Ogmore	SS8480 9270	1878	Situated in the village with the same name, in the outskirts of Maesteg. Completely surrounded by urban land. Sampling points were situated about 2m upstream and downstream from the mine discharge
Clyne	Clyne tributary, Tawe	SS5875 9430	1925	Located in a wooded area. Although close to the urbanised Dunvant, the land immediately surrounding the sampling area was exclusively forested. Sampling points were situated about 2m upstream and downstream from the mine discharge

[1] From Welsh coal mines website (http://welshcoalmines.co.uk)

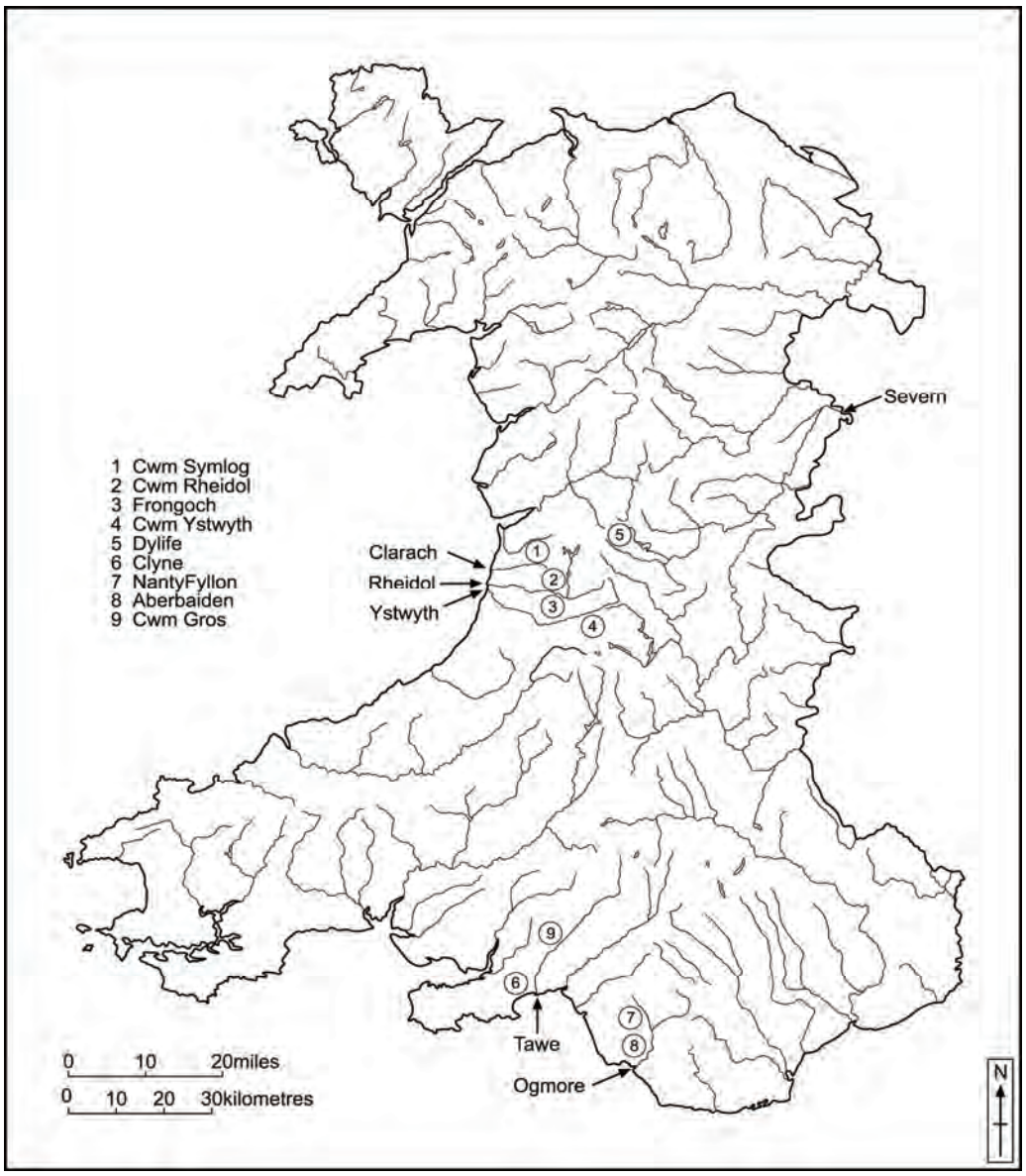


Figure 2.1 Location of study sites and associated catchment in Wales

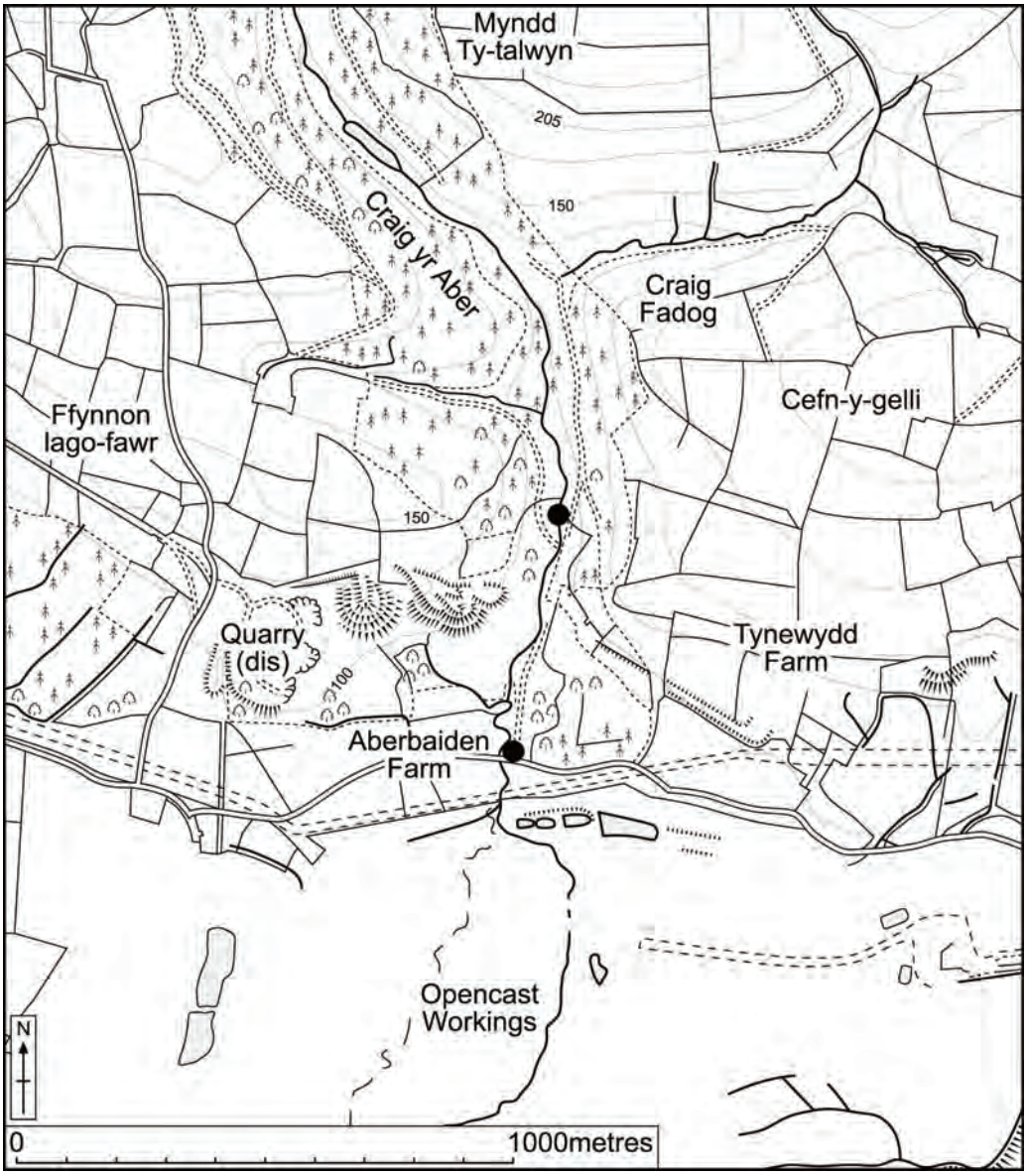


Figure 2.2 Map of the Aberbaiden coal mine study area showing upstream and downstream sampling points

Plate 2.1 Craig yr Aber downstream from Aberbaiden mine discharges showing typical orange ochre deposits (Source: Original)

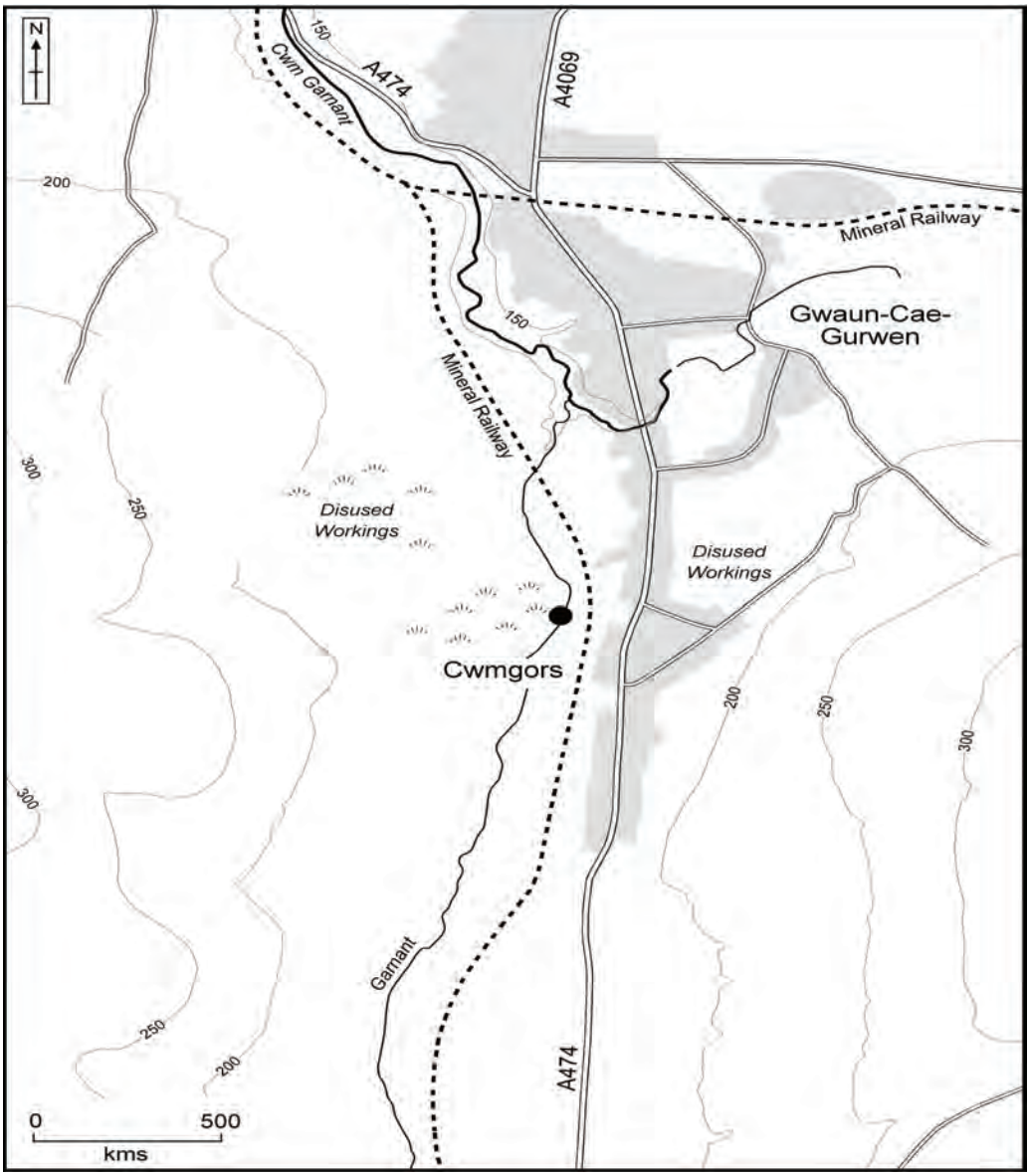


Figure 2.3 Map of the Cwm Gros coal mine study area showing sampling points upstream and downstream from the mine discharge (separated by 4m, due to map scale one dot represents both sampling points)

Plate 2.2 Cwm Gros coal mine discharge into Nant Melyn (Source: Original)

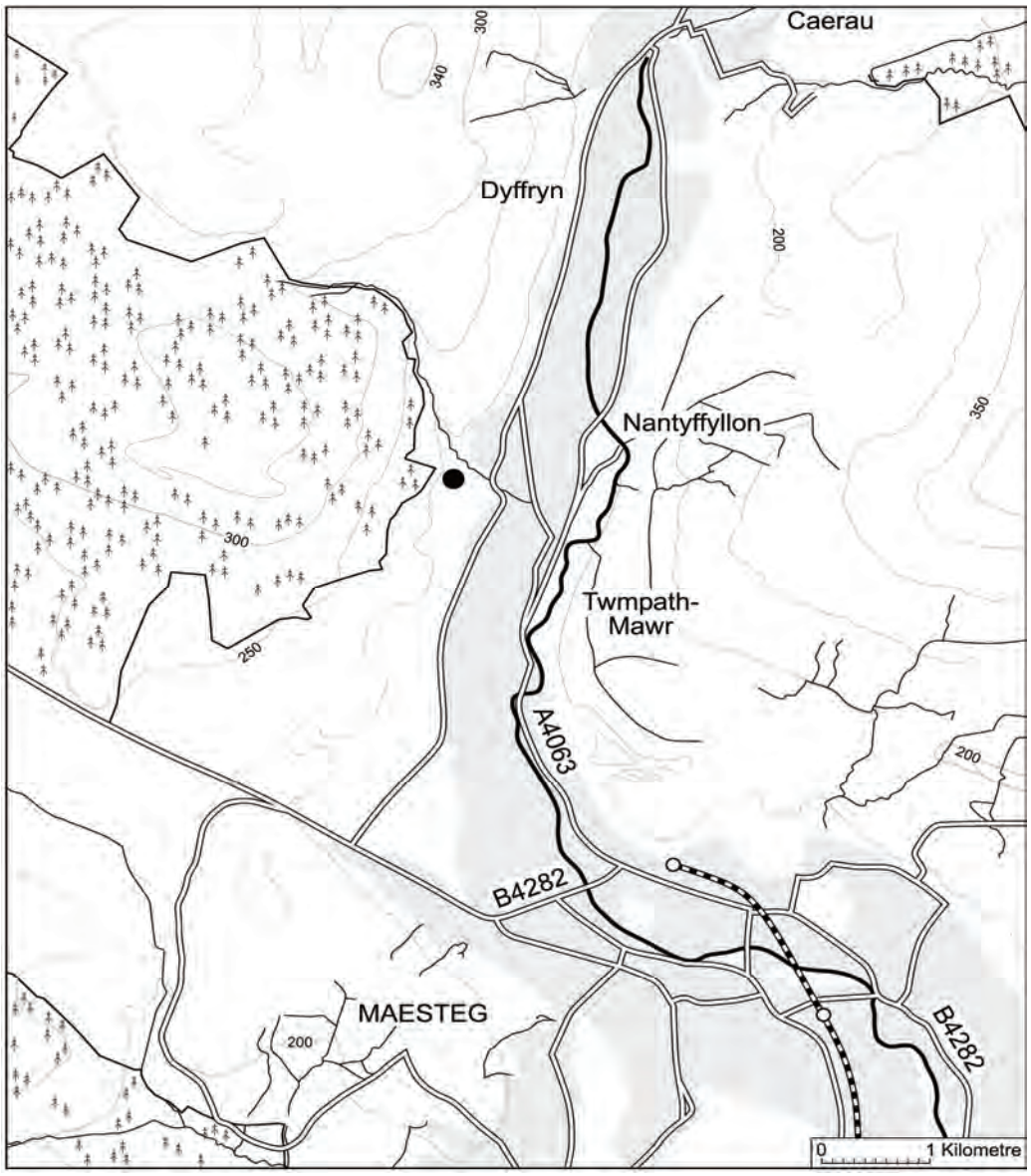


Figure 2.4 Map of the Nantyfyllon coal mine study area showing sampling points upstream and downstream from the mine discharge (separated by 4m, due to map scale one dot represents both sampling points)

Plate 2.3 Nantyfyllon coal mine discharge into Afon Nantyfyllon (Source: Original)

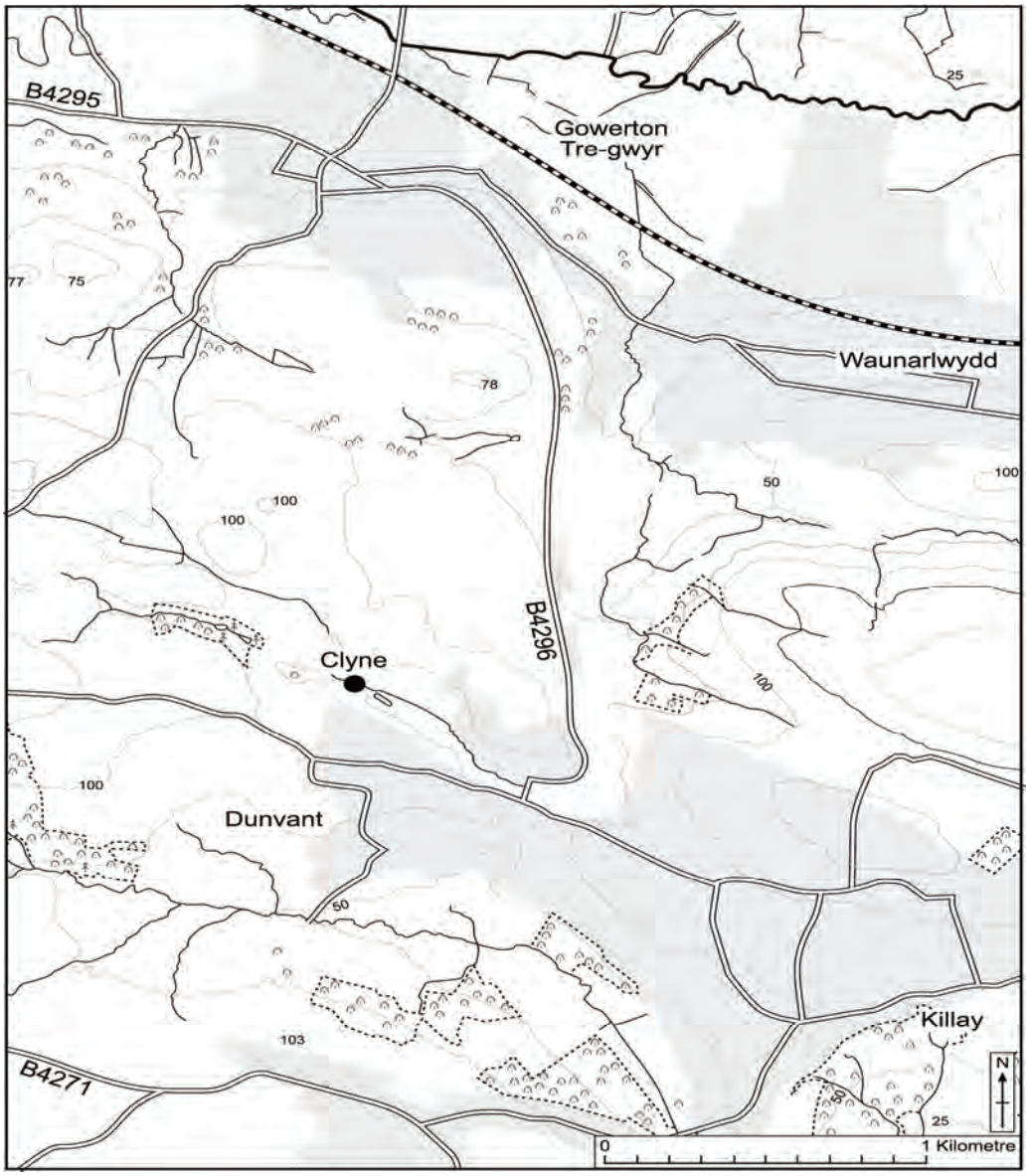


Figure 2.5 Map of the Clyne coal mine study area showing sampling points upstream and downstream from the mine discharge (separated by 4m, due to map scale one dot represents both sampling points)

Plate 2.4 Clyne coal mine discharge (Source: Original)

2.1.3 Streams impacted by abandoned metal mines

Mining in Mid Wales has taken place since Roman times, especially in Cardiganshire, a renowned area in the mining world (Bick, 1976). The peak of production in this area occurred during the 1840s, and the main ores worked were sphalerite or blende (zinc sulphite, ZnS), galena (lead sulphite, PbS), and chalcopyrite (copper and iron sulphite, $CuFeS_2$) (Bick, 1974). Pyrite (FeS_2), marcasite (FeS_2, similar to pyrite but with different crystal structure, making it more brittle), and traces of arsenopyrite (FeAsS), cobalate (CoAsS) and ullmanite (NiSbS) have also been recorded in the area (BGS, 1997). In the 1870s there was a decline in the mining industry due partly to price reductions in the world market, and many mines closed soon after that (Bick, 1974). This study considers some of the most important mines in the area; Frongoch was the most productive mine in Cardiganshire for sphalerite (Bick, 1976) and Dylife the biggest mine in West Montgomeryshire for galena and sphalerite (Bick, 1977).

The study sites were located on Palaeozoic mudstone, slate and siltstone (BGS, 1997) covered by shallow loamy or fine silty soils (Soil Survey of England and Wales, 1983). Some sites, namely Dylife, Cwm Symlog and Cwm Ystwyth, also had peaty soils (Soil Survey of England and Wales, 1983).

Similarly to the coal mines, the metal mines studied consisted of underground works. However, the metal mines presented numerous spoil heaps on the surface (Plates 2.5-2.8). Therefore, in order to include the underground mine drainage as well as the run off from

the spoil heaps in the study, the upstream and downstream sampling points were separated by approximately 1km. No major tributaries entered the stream within this reach. Similarly to coal mine impacted sites, sample site location only allows identifying local impacts within the streams, and downstream attenuation is not considered here as this is out of the scope of this study. .All the metal mine impacted study streams were located between 200m and 400m above sea level and their location is displayed in Figure 2.1 and Figures 2.6-2.9.

Table 2.2 Description of the four study sites impacted by metal mine drainage

Mine (Site's name)	Vein[1]	Water course and Catchment	Grid reference		Date of closure[2]	Description
			Upstream	Downstream		
Dylife	Galena and sphalerite	Twymyn, Clarach	SN8500 9400	SN8640 9400	1891	The mine was situated in a bleak mountainous landscape in the village with the same name, about 11km from Machynlleth. The surroundings of the downstream sampling points were characterised by bare stones forming spoil tips, the surrounding area was used as grazing land for sheep. Upstream and downstream sites were separated by approximately 1km.
Frongoch	Galena and sphalerite	Nant Cwmnewydion, Ystwyth.	SN7170 7370	SN7110 7410	1910	Frongoch mine was located in agricultural land, and the sampling area was surrounded by grazing fields where cattle and sheep were found.. The upstream site was located downstream from a boggy area, and about 1km downstream was found the second sampling point. The stream was shaded by oak and maple trees at the downstream site.
Cwm Ystwyth	Galena, sphalerite, pyrite and chalcopyrite	Yswyth.	SN8140 7480	SN7995 7430	1921	Cwm Ystwyth mine is located about 2km from the village with the same name. It was a big mine which caused a high visual impact. Spoil tips and abandoned buildings covered the whole northern slope of the valley. The southern part of the valley was used for grazing. The upstream site was located by Tymawr farm and the following site about 1km downstream to include the impact of the run off of the spoil tips.
Cwm Symlog	Sphalerite, galena, chalcopyrite, marcasite and arsenopyrite	Symlog, Ystwyth	SN7030 8375	SN6910 8390	1901	Cwm Symlog mine was located by the village with the same name. It was surrounded by coniferous forests and grassland. Numerous spoil tips and abandoned buildings could be found in this site. The downstream sampling point was separated by 1km from the upstream to include the effect of all the spoil tips found in the site.

[1] From British Geological Society (BGS) (1997) "Geology of the country around Llanilar and Rhyader"

[2] From Bick (1974, 1975, 1977) "The old metal mines of Mid Wales" volumes 1,2,4

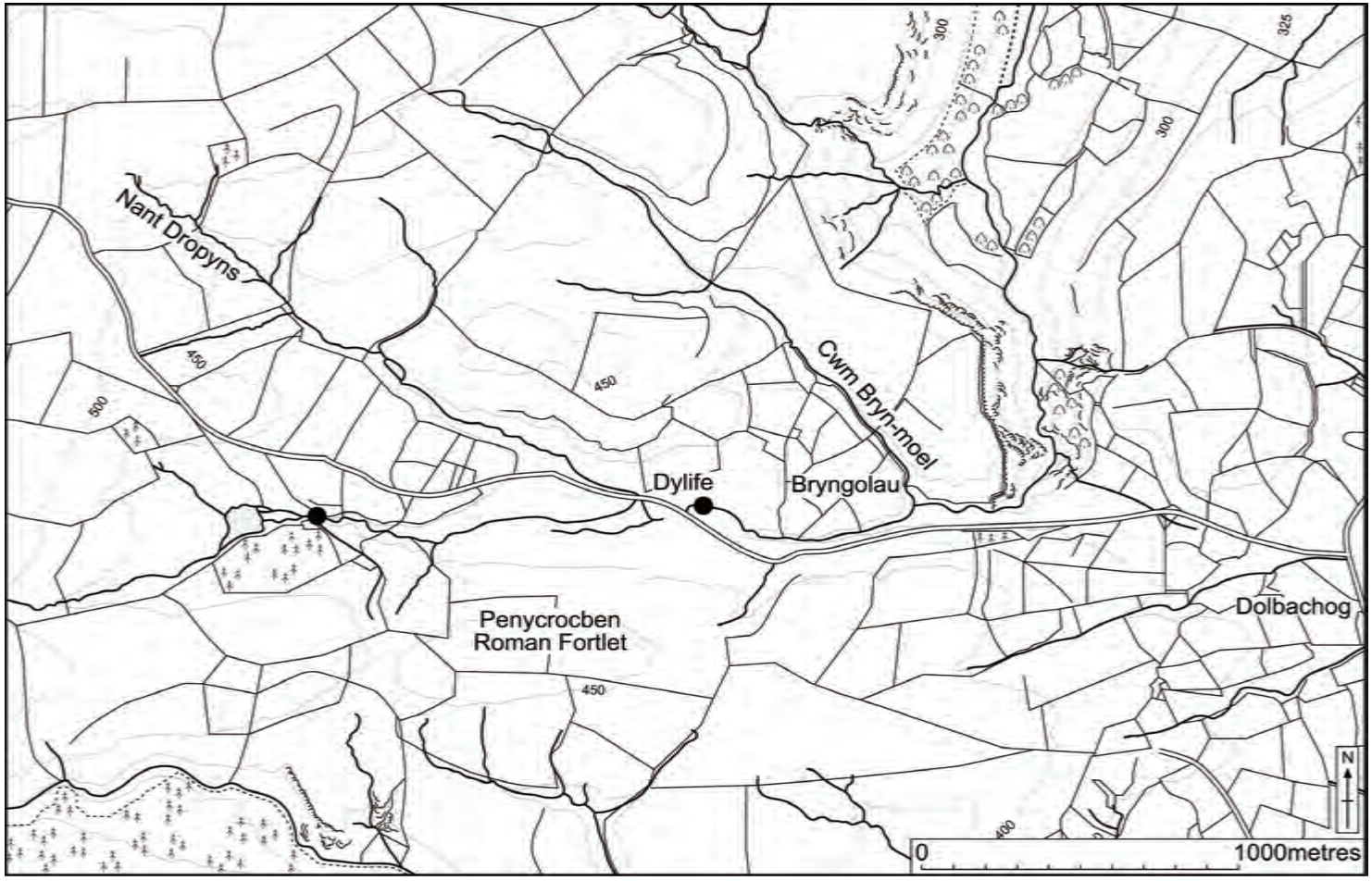


Figure 2.6 Map of the Dylife metal mine study area showing upstream and downstream sampling points

Plate 2.5 Spoil heaps around Afon Twymyd downstream from Dylife mine (Source: Original)

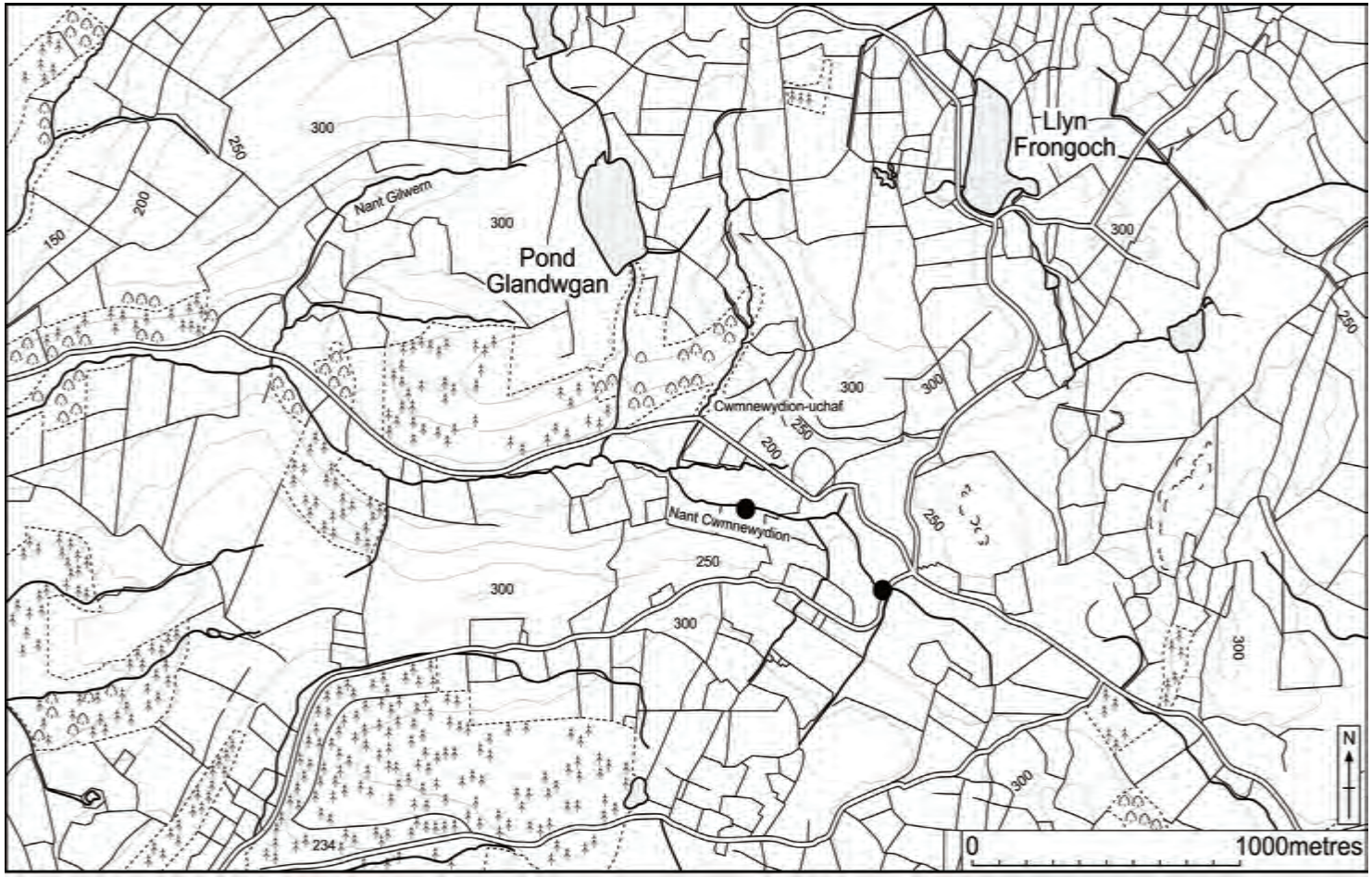


Figure 2.7 Map of the Frongoch metal mine study area showing upstream and downstream sampling points

Plate 2.6 Spoil heaps and abandoned buildings at Frongoch mine site (Source: Original)

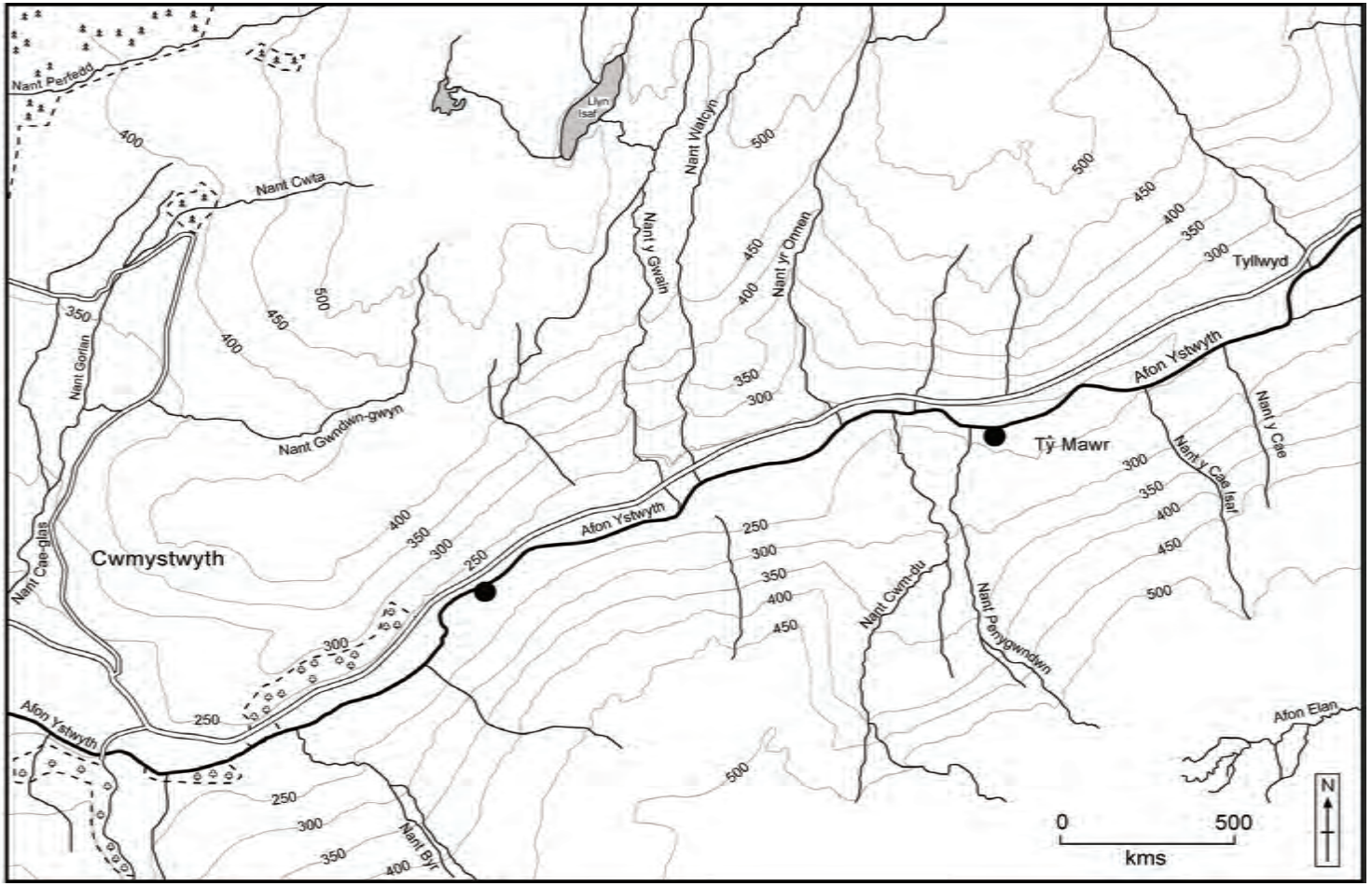


Figure 2.8 Map of the in Cwm Ystwyth metal mine study area showing upstream and downstream sampling points

Plate 2.7 Spoil heaps located by Afon Ystwyth downstream from Cwm Ystwyth mine (Source: Original)

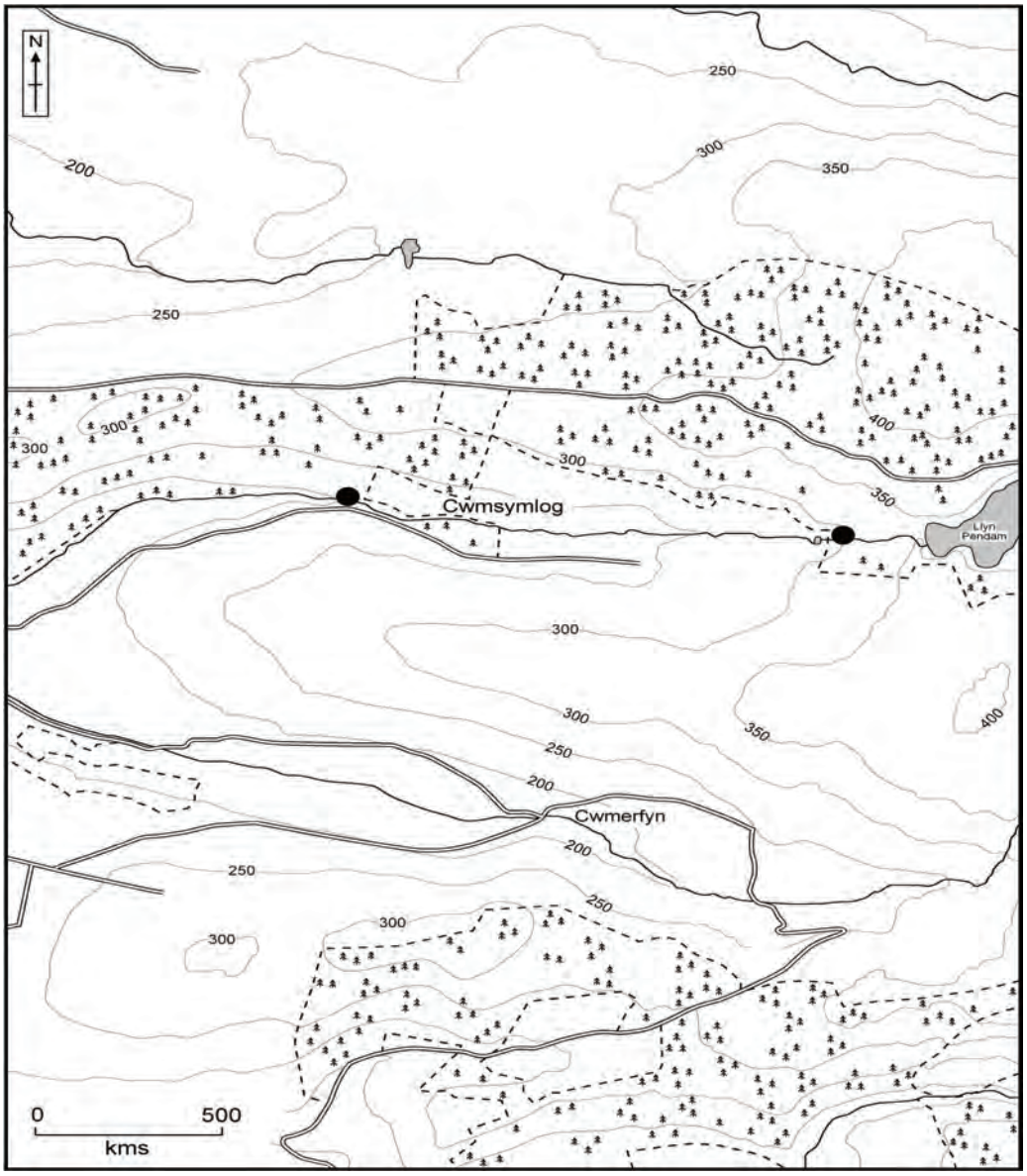


Figure 2.9 Map of the Cwm Symlog metal mine study area showing upstream and downstream sampling points

Plate 2.8 Spoil heaps in the abandoned mine site of Cwm Symlog (Source: Original)

2.2 Water sampling and analysis

For the study of eight Welsh streams impacted by abandoned mine drainage (Chapters 3-5), water and sediment samples were taken upstream and downstream from the polluting mines 4 times a year (seasonally) at the same time as macroinvertebrates were collected. Upstream and downstream sites were sampled within the same hour, and different streams were sampled within the same week. Sampling regime used in Chapters 6 and 7 is detailed in the relevant sections.

2.2.1 Field measurements

Some hydrochemical measurements were taken in the field in order to characterise the quality and quantity of stream water. Table 2.3 shows the variables measured and the instruments used. The Ultrameter^{TM} and the YSI 550A were calibrated on arrival at each site.

Table 2.3 Hydrochemical variables measured on site and the instrument used

Variable	Instrument
pH Conductivity Total dissolved solids	UltrameterTM model 6P
Flow	SENSA RC2
Dissolved oxygen Temperature	YSI 550A

One reading of each variable was made except for flow, where 5 measurements were taken. Flow was measured at five evenly spread points through the channel and at 60% depth,

where the surface fast flow averages out against the slower bed flow (Geopacks, 2002). The method used to calculate stream discharge consisted of dividing the stream cross-section into 6 columns and calculate the average discharge (l/s) in each column (Equation 2.1), the discharge in the whole cross-section corresponds to the sum of each individual discharges (Geopacks, 2002).

$$Q_2 = A_2 * v_{1,2} \qquad \text{Equation 2.1}$$

Where Q_2 is the discharge in column 2, A_2 is the area in column 2 and $v_{1,2}$ is the mean velocity between measuring points 1 and 2.

2.2.2 Suspended solids

One 500ml water sample was taken in the field and transported to the laboratory to be filtered through 0.45µm cellulose nitrate filter, as suspended solids (SS) are defined as those sediments bigger than 0.45 µm (Fabuss and Fabuss, 1974). Filters were previously dried at 80ºC and weighed. Once the sample was filtered, the filter was dried under the same conditions and weighed again. Suspended solids were calculated by the difference in weight, taking into account the sample volume as shown in Equation 2.2:

$$SS = (W_2 - W_1)/V \qquad \text{Equation 2.2}$$

Where,

SS: Suspended solids (mg/l);

W_2: Filter dry weight after filtering (mg);

W_1: Filter dry weight (mg);

V: Sample volume (l).

2.2.3 Major ion analysis: Dionex and ICP-MS analysis

One 500ml sample was taken in the field and immediately filtered through 0.2 µm cellulose nitrate filter paper. Filtering of the sample through a 0.2 µm filter eliminated solids and colloids present in the water which may have damaged the equipment used for analysis, caused interferences or continued reacting in the bottle (APHA et al., 1992). Two 50ml vials were filled with the filtered water. One of the 50ml samples was acidified in the lab to 4% volume of nitric acid and analysed for heavy metals using ICP-MS. The other 50ml vial was directly analysed for nitrate, sulphate, chloride and phosphate using ion chromatography (Dionex).

2.2.3.1 Dionex – Ion chromatography

Dionex ion chromatography was used to analyse major ions in water. For the present study, ICS 2000 with a gradient method, was used for nitrate, phosphate, sulphate and chloride determination (detection limits displayed in Table 2.4). The analyses were undertaken using standards of 0.25, 1, 2.5, 5, 10 and 20 ppm for each of the mentioned ions. An independent

check standard of 5ppm was used to check for drift during the run. The run was considered acceptable if the data were within 5% of the expected concentration (between 4.75-5.25ppm).

Table 2.4 Anion detection limits in Dionex analysis

Anion	Detection limit (ppm)
Chloride	0.06
Nitrate	0.04
Sulphate	0.07
Phosphate	0.06

Samples with concentrations higher than 20ppm were diluted to fit within the calibration rank. As the samples are characteristically high in heavy metal concentrations, they were filtered beforehand with Dionex OnGurad IIM cartridges, which contain iminodiacetate resine in the ammonium form, which removed transition metals from the sample (Dionex, 2004). Metals needed to be removed prior to analysis as they may damage the equipment.

2.2.3.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS was used to determine cations in the stream water samples: iron, manganese, zinc, cadmium, copper, lead, nickel, calcium, sodium, magnesium and potassium (detection limits displayed in Table 2.5). The Agilent 7500 ce instrument used for the analysis performed rapid multi-element determinations in liquid samples, identifying cations within the range of ppm-ppt. The instrument applied octopole ‘collision cell technology’ using

helium and hydrogen gases to eliminate spectrally based interferences. The instrument was run separately for the analysis of trace metals (ppb) and major cations (ppm) due to the difference in concentration. For trace metals, the instrument was calibrated with 0ppb, 50ppb, 100ppb and 5000ppb standards. For major cations 0ppm, 2ppm, 10ppm and 40ppm standards were used for calibration. In all cases, an independent check standard of 1ppm was used to check for drift during the run. The run was considered acceptable if the data were within 5% of the expected concentration (between 0.75-1.25ppm).

Table 2.5 Cation detection limits in ICP-MS analysis

Cation	Detection limit (ppm)
Iron	0.005
Manganese	0.005
Zinc	0.005
Cadmium	0.005
Copper	0.01
Lead	0.005
Nickel	0.05
Calcium	0.02
Sodium	0.02
Magnesium	0.02
Potassium	0.5

2.3 Sediment sampling and analysis

Small quantities (c. 200g) of approximately the first 3cm of sediment (including the armour layer) were sampled at each site and transported to the laboratory in plastic bags. Homogenised samples were wet sieved and the fraction smaller than 63µm was oven dried at 80ºC and kept for analysis in plastic bags (Brown, 1977), as fine grain sediments often have higher trace metal concentrations (Ladd et al., 1998).

The aqua regia method (AR) was used for sediment digestion and trace metal extraction, which involves a 3:1 mixture of concentrated hydrochloric acid (Analar) and concentrated nitric acid (Analar). These acids react to give aqua regia:

$$3HCl + HNO_3 \longrightarrow 2H_2O + NOCl + CL_2$$

AR digestion is considered an effective method for the extraction of most metals (Wilson and Pyatt, 2007). 0.5 grams of sediment was weighed into a digestion tube with 5ml of aqua regia (3:1 HCl and HNO_3) and left in the fume cupboard at room temperature for 16 hours. Subsequently, samples were heated at 80˚C for 2 hours and then allowed to cool to room temperature.

Samples were filtered through Whatman No1 filter papers into a 100ml volumetric flask. 1ml of 10% KCl_2 was added as an ionisation suppressant and the dilution was made up to 100ml. Samples were kept at 5˚C in the dark before analysis using atomic absorption spectrophotometry

2.3.1 Trace metal analysis: Atomic Absorption Spectrophotometry (AAS)

The digest obtained from the sediments was analysed for iron, cadmium, lead, manganese, copper, nickel and zinc using an Analyst 300 atomic absorption spectrometer (AAS) (detection limits are displayed in Table 2.6). A deuterium arc lamp was used to correct for background (the absorbance caused by non-atomic species in the atom cloud) during zinc analysis.

Different standard concentrations were used for different elements depending on their absorbance graph (Table 2.7). Samples with concentrations that exceeded those of the standards were diluted to fit within the calibration rank.

Table 2.6 Trace metal detection limits in AAS analysis

Element	Detection limit (ppm)
Iron	0.03
Cadmium	0.04
Lead	0.04
Manganese	0.01
Copper	0.01
Nickel	0.01
Zinc	0.02

Table 2.7 Calibration standards concentrations used for AAS analysis

Element	Standard concentrations (ppm)
Iron	2, 4, 6
Cadmium	1, 2, 3
Lead	5, 10, 20
Manganese	1, 2, 3
Copper	2, 4, 6
Nickel	3, 6, 9
Zinc	2, 3, 4

2.4 Macroinvertebrate sampling and identification

2.4.1 Macroinvertebrate sampling

Four Surber samples were collected at each sampling point (see Section 2.1 for details on sample locations). The Surber sampler used had a 250µm mesh and covered an area of $0.1m^2$. Samples were preserved in 10% formaldehyde solution for summer 2006 sampling season and in neat ethylene glycol for the rest of the campaign. They were kept refrigerated at 5ºC until processing. In the laboratory, samples were rinsed with water through a 250µm mesh sieve, sorted and preserved in 70% Industrial Methylated Spirit until identification.

2.4.2 Macroinvertebrate identification

Freshwater Biological Association keys were used to identify Plecoptera (Hynes, 1977), Trichoptera (Wallace et al., 1990, Edington and Hildrew, 1995) and Ephemeroptera (Elliot and Humpesch, 1983) to species level. Chironomidae were subsampled when found in large numbers: 25 Chironomidae per sample (100 per site) were identified, and total numbers

were deduced from this. Prior to identification, chironomids were washed in KOH for 20 minutes to dissolve the soft parts inside the head capsule to facilitate their identification. Subsequently, they were fixed to slides with Dimethyl Hydantoin Formaldehyde and identified to species level where possible with the aid of several taxonomic keys (Cranston, 1982, Wiederholm, 1983, Brooks et al., 2007). Other macroinvertebrates were identified to family or genus level following Croft (1986) “Key to major groups of British freshwater invertebrates”.

2.5 Statistics

2.5.1 Upstream and downstream comparisons

One of the main objectives of this project was to identify any differences within streams due to abandoned mine drainage discharging into them. The first approach taken was a paired comparison upstream and downstream of the mine discharge. This was applied to both the chemical and ecological data. As the data were not normally distributed, Wilcoxon signed rank test, a non-parametric test, was chosen. This is based on the rank order of the differences between two groups rather than the actual value of the difference (Harrad et al., 2008).

Analysis of variance (ANOVA) was used to assess if there were differences between the means of several populations, based on samples taken from each population. One-way ANOVA assesses how likely it is that all the samples come from populations with the same

mean, considering the variation between the means and between measurements within samples (Townend, 2005). One-way ANOVA was used to estimate differences in standardised measurements (e.g. decomposition) upstream and downstream from the mine discharge. SPSS version 15.0 was used for both Wilkcoxon signed rank test and One-way ANOVA test.

2.5.2 Univariate statistics using Brodgar

Univariate statistical analyses were carried out using the Brodgar software package. Two main functions were used: exploration statistics and Generalised Additive Modelling (GAM).

2.5.2.1 Data exploration

Most statistical analyses assume that the data comply with several assumptions: homogeneity, normality and no correlation between explanatory variables (Zuur et al., 2007). If these assumptions are not met, the results of the analyses may be invalid. Exploration statistics offered by Brodgar were used in order to identify data that did not fit these assumptions:

1. Cleveland dotplots were used in order to identify outliers or heterogeneity in the data set. If these were found, and they were real values (not analytical errors), data were transformed. If transformation did not solve the problem, outliers were discarded from the analysis.

2. Quantile-Quantile plots (QQ-plots) were used to establish the most appropriate type of transformation for the data set. QQ-plots compare the distribution of a given variable to the Gaussian distribution. The function produces graphs where the data were displayed as un-transformed, square transformed, square root transformed and log transformed. The graphs were used to visually identify the best transformation option. If the resulting plot is roughly a straight line, the data were considered to be normally distributed (Zuur et al., 2007).

3. Pariplot show multiple pair-wise scatterplots in one graph and were used to detect relationships between variables and collinearity (Zuur et al., 2007). When this was encountered, one variable was selected from the covarying pair that was thought to represent the best and most ecologically sensible outcome. This also helped to reduce the pool of variables used in the analysis in order to simplify interpretation.

As the pool of environmental variables was large in comparison to the pool of response variables (due to difference in effort required to obtain these two types of data), those variables that did not significantly change downstream from the mines were not considered for further statistical analysis.

2.5.2.2 Generalised Additive Modelling

Generalised additive modelling (GAM) uses smoothing curves to model the relationship between the response variable and the explanatory variables, allowing for non-linear model (Fox, 2002). Non-linear relationships between response and explanatory variables are common in ecology, hence GAM was used in several parts of this study.

Poisson distribution with log link was used in this study, because the data did not fulfil the homogeneity assumption when using additive modelling (Zuur et al., 2007). The best fit model was chosen with forward selection based on AIC values (Fox, 2002), and misspecifications were checked by plotting the residuals of the model against the original explanatory variables and a visual inspection of their pattern to look for homogeneity issues (Zuur et al., 2007). For each response variable, a model with the smaller AIC and with no pattern on the residuals graph was chosen.

2.5.3 Multivariate statistics using CANOCO

Ordination analysis can be used to isolate 1, 2 or 3 axes (ordination axes) that explain the greatest variability in community composition for a set of samples (Ter Braak and Verdonschot, 1995). The axes can be related to one particular factor (such as pH), or, more often, a combination of several factors (Leps and Smilauer, 2007). Ordination was used to summarise relationships between predictors (environmental data) and multiple response variables (species data), hence, constrained ordination was chosen. Constrained ordination or direct gradient analysis identifies the variability in species composition that can be

explained by the measured environmental variables (Wollenberg, 1977). In this analysis, the ordination axes are weighted sums of environmental variables and represent the direction of the greatest data set variability that can be explained by the environmental variables (Wollenberg, 1977). The fewer environmental variables used in the analysis, the stricter is the constraint; as a rule of thumb, the number of environmental variables must not exceed the number of samples minus two (Leps and Smilauer, 2007).

Ordination can be based on linear or unimodal species response to the environmental gradient. The selection of one of the methods can be undertaken by a technique described in Leps and Smilauer (2007): Detrended canonical correspondence analysis (DCCA) is run with the whole set of data that is being studied. The gradient length obtained with this analysis measures the beta diversity in the community composition along the ordination axes (Leps and Smilauer, 2007). Generally, when the longest gradient is shorter than 3, Redundancy Analysis (RDA) can be adopted; if it is longer than 4, Canonical Correspondence Analysis (CCA) can be used. Where the longest gradient length is between 3 and 4, the most significant method is chosen using a Monte Carlo permutation test (see below).

The significance of constrained ordination analysis can be tested using Monte Carlo permutation test. In this test, the null hypothesis states that species data distribution is independent of the explanatory data (environmental variables) (Manly, 1997). The test consists in randomly assign environmental variables to individual samples of species

composition, undertake an ordination analysis with this permuted data set, and calculate the value of the test-statistic with the following formula:

$$p = \frac{n_x + 1}{N + 1}$$

Where n_x is the number of permutations where F-statistic is as large or larger than in the analysis of the original data, and N is the total number of permutations. Therefore, the smaller the n_x, the smaller is the value of the test statistic, rejecting the null hypothesis.

Several statistical tests can be run following basic ordination analysis. In this study forward selection of explanatory variables was used to find a subset of variables that represents the relationship between species and the environment (Leps and Smilauer, 2007). Monte Carlo permutation tests (Manly, 1997) were used to assess the significance of each explanatory variable added to the ordination model. Variance partitioning (Borcard et al., 1992) was used to discern between the effect of two sets of explanatory variables (e.g. water and sediment chemistry). The percentage of species variation explained by the first set of variables is estimated from the sum of eigenvalues using these variables as *environmental variables* and the second set of explanatory variables as *covariables* in the ordination analysis in CANOCO (Legendre and Legendre, 1998). Similarly, the percentage of species variation explained by the second set of explanatory variables can be estimated from the sum of eigenvalues using these variables as *environmental variables* and the first set as *covariables* (Legendre and Legendre, 1998).

Ordination diagrams were used to visualise the output of the analysis and aid on the study of the relationship between environmental variables and species composition. Basic ordination diagrams can display the distribution of explanatory variables, samples and species data (for example, Figure 3.3 in Chapter 3), providing visual information on similarity (proximity) between samples, intensity and direction of the effect of environmental variables (arrows) and distribution of samples and species along the environmental gradient (ordination axes). Alternatively, sample characteristics (e.g. species richness and abundance) can be plotted together with environmental variables for further study of the relationship between species data and the environment. CANOCO offers the option of displaying these diagrams with smoothed data, using various methods such as GAM (see above) (for example, Figure 4.1 in Chapter 4).

3 CHEMISTRY OF STREAMS IMPACTED BY MINE DRAINAGE

3.1 Introduction to mine drainage hydrochemistry

Extraction of mineral ores affects the hydrology of catchments in two main ways, influencing the quality and quantity of water. The processes causing these changes occur during the whole mining life cycle and include: extraction, mineral processing, dewatering of the worked area, leaching from waste rock piles and tailing dams, flooding of tunnels after cessation extraction, and discharge of polluted water (Younger et al., 2002). In the long term, the most important impacts caused by abandoned mines are diffuse runoff from spoil tips and point discharges of mine drainage, which lead to surface and ground water pollution. This thesis focuses upon the chemical impact of mine pollution on the ecology of receiving streams and therefore processes governing the quantity of water are not covered in this chapter.

Abandoned collieries typically produce acid mine drainage, which is high in total dissolved solids, principally metals and sulphates, has low pH and high suspended solids (Herricks and Cairns, 1977, Parsons, 1977) (Table 3.1). In contrast, abandoned metal mines normally load the water with heavy metals and sulphates, but not acidity and suspended solids (Younger at al., 2002) (Table 3.3). These differences are due to the chemistry of the ores present in the mines: the main mineral found in coal is pyrite (FeS_2),

whereas sphalerite (ZnS) and galena (PbS) are examples of typical ores worked in metal mines (Bick, 1974). This section discusses the processes involved in coal and metal mine drainage formation as well as their effect on the chemistry of receiving water courses.

3.1.1 Water and sediment pollution by coal mine drainage

The processes involved in coal mine drainage formation have been widely studied (Langmuir, 1997, Smith, 1999, Younger et al., 2002). The main reactions are described through Equations 3.1 to 3.4.

Pyrite (FeS_2) is the main mineral present in coal. When it is weathered in the presence of oxygen, redox reactions transform sulphur and iron into soluble sulphate and ferrous iron.

$$2FeS_2(s) + 7O_2(aq) + 2H_2O \longrightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \qquad (3.1)$$

If there is enough oxygen in the water the reaction can go farther, oxidising the ferrous iron to the ferric form.

$$2Fe^{2+} + 1/2O_2 + 2H^+ \longrightarrow 2Fe^{3+} + H_2O \qquad (3.2)$$

Ferric iron can also be the oxidising agent in pyrite weathering, producing ferrous iron and acidity.

$$14Fe^{3+} + FeS_2(s) + 8H_2O \longrightarrow 2SO_4^{2-} + 15Fe^{2+} + 16H^+ \qquad (3.3)$$

Table 3.1 Chemical characteristics of water draining from several collieries and some streams impacted by coal mine drainage

River	pH	Fe (ppm)	Mn (ppm)	Cu (ppm)	Cd (ppm)	Ni (ppm)	Cr (ppm)	Zn (ppm)	Pb (ppm)	SO_4^{2-} (ppm)	Reference
Matsuo mine drainage (Japan)	2.3	237									Sasaki et al. 2005
Drift mine discharge (Wales, UK)	7.8	15.79	3.12	0.09				1.30		612	Scullion and Edwards 1980
Avoca mine drainage (Ireland)	2.67	996		185	0.916			229		10203	Gray 1998
Kimblesworth mine drainage (UK)	6.95	1.87	0.63							307.7	Jarvis and Younger 1997
Bulhouse mine drainage (UK)	5.61	62	11.5	0.019	0.019	0.046	0.021	0.156	0.003	231.5	Bermingham et al. 1996a
River Akagawa (Japan)	3.7	10	0.87	4.5	3.6	96	22	62	14		Sasaki et al. 2005
Taff Bargoed (Wales, UK)	7.8	1.80	0.20	0.05				0.07		110	Scullion and Edwards 1980
Avoca River (Ireland)	5.8	1400		60	610			640		28	Gray 1998
Rio Tinto (Spain)	2.70	489	25	55	0.274	0.493	0.06	55	0.508	2583	Canovas et al. 2008
Slippery Rock Creek (Pennsylvania, USA)	6.3	0.4	4.10					0.07		255	DeNicola et al. 2002
Racoon Creek (Ohio, USA)	3.2		3.4							411	Dsa et al. 2008
Indian Creek (Pennsylvania, USA)	6.0	1.5								65.8	Herricks and Cairns 1972
River Don (UK)	6.3	11.72	3.05	0.005	0.001	0.045	0.0005	0.007	0.005	65.4	Bermingham et al. 1996a

Under acidic conditions (pH<3) Fe^{2+} oxidation to Fe^{3+} (Equation 3.2) is enhanced. As a result, pyrite oxidation is accelerated, as this reaction is faster when Fe^{3+} rather than oxygen is the oxidising agent (Equation 3.3) (Equation 3.1) (Langmuir, 1997). Such low pH values (pH<3) are rarely found in receiving streams (Table 3.1) due to natural buffering capacity of most water bodies (e.g. contact with carbonate bedrock) or to dilution with neutral waters.

Acidophilic bacteria such as *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* are known to catalyse and accelerate pyrite oxidation at slightly acidic conditions, their optimal pH range being 5-8 (Younger et al., 2002; Langmuir, 1997). These pH values are commonly found in streams polluted by coal mine drainage (Table 3.1), suggesting that biotic reactions dominate in natural environments.

Fe^{3+} can further react in the stream water if pH increases to values above 5, where it is highly insoluble and precipitates as iron oxyhydroxide (ochre) (Equation 3.4) (Kimball et al., 1995, Parkman et al., 1996, Jain and Ram, 1997, Langmuir, 1997, Younger et al., 2002, Stillings et al., 2008), covering the stream bed with orange precipitate (Plates 2.1-2.4, Chapter 2). This pH range (pH>5) is common in streams impacted by coal mine drainage (Table 3.1), thus precipitation and accumulation of ochre on the river bed is one of the main pathways transporting iron from the water column into the sediments.

$$2Fe^{3+} + 6H_2O \longleftrightarrow 2Fe(OH)_3(s) + 6H^+ \qquad (3.4)$$

Table 3.2 Trace metal concentrations from sediments collected in streams impacted by coal mine drainage pollution

River	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Ni (mg/kg)	Cr (mg/kg)	Zn (mg/kg)	Pb (mg/kg)	Reference
Ely Creek (VA, USA)	4014.6	71.4	3.035	0.270	4.18	4.423	14.600		Cherry et al. 2001
Avoca River (Ireland)	7.7		593				832		Herr and Gray, 1997
Slippery Rock Creek (Pennsylvania, USA)	51000	27900					300		DeNicola et al. 2002
Federal Creek (Ohio, USA)	12481	4706.8	0.80			0.2	9.8	3.5	Dsa et al. 2008

Sediments collected in streams impacted by coal mine drainage usually have high concentrations of iron, but often also other metals (Table 3.3). This is due to co-precipitation of other metals present in the water column with iron oxyhydroxide (Smith, 1999) (See section 3.1.2).

Tables 3.1 and 3.2 summarise the chemical characteristics of coal mine drainage and streams impacted by it discussed in this section. The pH in receiving streams vary from values as low as 2.7 to up to 7.8, which depends on the natural buffering capacity of the impacted stream. The most abundant metal in these streams is often dissolved iron, which can be found in concentrations greater than 100ppm, one hundred times the EQS established for iron of 1ppm (Table 1.1, Chapter 1). Iron is found in greatest concentrations when waters are acidic, due to higher solubility of the cation. In sediments collected from these streams, iron is the most abundant metal, which can reach concentrations of 51000mg/kg. The tables also show that other metals can be found in the stream water and sediment, but these depend on the characteristics of the ore worked in the mine.

3.1.2 Water and sediment pollution by metal mine drainage

When sulphide minerals other than pyrite are subject to weathering, they do not necessarily produce acidity, yet metal ions and SO_4^{2-} are still released into the environment (Younger et al., 2002). Some examples of these are sphalerite (ZnS) (Equation 3.5), galena (PbS) (Equation 3.6) and chalcopyrite ($CuFeS_2$) (Equation 3.7); which release zinc, lead, copper and iron.

$$ZnS + 2O_2(aq) \longrightarrow Zn^{2+} + SO_4^{2-} \quad (3.5)$$

$$PbS(s) + 2O_2 \longrightarrow Pb^{2+} + SO_4^{2-} \quad (3.6)$$

$$CuFeS_2 + 2O_2(aq) \longrightarrow Cu^{2+} + Fe^{2+} + 2SO_4^{2-} \quad (3.7)$$

Table 3.3 displays some examples of metal concentration and pH values in metal mine drainage and water composition of streams affected by metal mine pollution. Zinc (83-1480ppb) and lead (0.4-20200ppb) are typically the most abundant metals in these streams. These streams typically have pH much higher than those impacted by coal mine drainage due to the reactions discussed above. However, Afon Rheidol mine drainage is highly acidic (pH=2.7) (Fuge et al., 1991). Even though iron concentrations were not measured in Fuge et al. (1991) study, sphalerite worked at Cwm Rheidol mine is known to be rich in pyrite (Bick, 1974), which could be responsible for drainage acidification (Chapter 8).

Table 3.3 Chemical characteristics of water draining from several metal mines and some streams impacted by metal mine drainage

River	pH	Fe (ppb)	Mn (ppb)	Cu (ppb)	Cd (ppb)	Ni (ppb)	Cr (ppb)	Zn (ppb)	Pb (ppb)	SO_4^{2-} (ppm)	Reference
Cwm Rheidol mine discharge (Wales, UK)	2.9			49	74	555		55000	26	643.5	Fuge et al. 1991
River Gaula (Norway)	6.77			11				83			Arnekliev and Storset 1995
Tarapaya River (Bolivia)				13	5.0			601	56.0		Smolders et al. 2002
Pilcomayo River (Bolivia)				14	0.76			238	28.5		Smolders et al. 2002
Mosquito Creek (Colorado, USA)	8.3			BD	BD			168			Kiffney and Clements 1996
Jack Creeck (Montana, USA)		33		41	9.0			942	0.5		Farag et al. 2006
Saxbäcken river (Sweden)		390		5.9	1.23			1480	0.4		Malmqvist and Hoffsten 1999
Estanda stream (Basque Country, Spain)	8.23	130	27970	2570	530		3160	320	20200		Marques et al. (2003)
Afon Rheidol (Wales, UK)	6.1				1.5	3.15		269.5	6.3	7.1	Fuge et al. 1991
Afon Ystwyth (Wales, UK)	4.1				1.0	3.0		307	68		Fuge et al. 1991
Eagle River (Colorado, USA)	8.10	600	615	BD	BD			107			Hill et al. 2000

BD: below detection limit

Table 3.4 Trace metal concentrations in sediments collected from streams impacted by metal mine drainage

River	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Ni (mg/kg)	Cr (mg/kg)	Zn (mg/kg)	Pb (mg/kg)	Reference
Jack Creek (Montana, USA)			180	4.2			490	190	Farag et al. 2006
Boulder River Montana, USA)			110	3.2			430	80	Farag et al. 2006
Tarapaya River (Bolivia)			296	107			9058	902	Smolders et al. 2003
Pilcomay River (Boliva)			158	31.7			4513	603	Smolders et al. 2003
Oiartzun River (Basque Country, Spain)	51800	1850	130	23.0	50	66	3080	870	Sanchez et al. 1994
River Dommel (Belgium)			43.24	84.17			1760.21	384.33	De Bisthoven et al. 1998
River Dijle (Belgium)			24	1.68			240.36	81.44	De Bisthoven et al. 1998
East fork River (Colorado, USA)	8014	824	10.5	0.84			935	88.9	Ruse et al. 2000
Arkansas River (Colorado, USA)	30400	825	157.0	2.97			2374	779.0	Ruse et al. 2000

Unlike Fe^{3+}, these other metals do not typically form oxides which precipitate out of the water column, although they are often found in stream bed sediments (Table 3.4). Thus, they follow other pathways of transfer from the water column into the river bed. Trace metals are known to be adsorbed by suspended sediments present in the water column, which subsequently settle in the river bed, and remove them from solution (Smith, 1999).

Sorption reactions depend on many factors:

a) pH is one of the most important factors, with adsorption of metal and other cations increasing with a rise in pH (Ghanem and Mikkelsen, 1988, Jain and Ram, 1997).

b) Sorbate identity and concentration, and presence of competing sorbates. Cations have different affinities for the sorbent, trace metal sorption capacities are know to follow the order: Hg>As>Pb>Cu>Zn>Ni>Cd (Jean and Bancroft, 1986, Sidle et al., 1991, Jain and Ram, 1997, Smith, 1999).

c) Formation of solution complexes. Mineral oxides have high affinity for dissolved phosphorus (Ivorra et al., 2002, Adler and Sibrell, 2003, Wei et al., 2008). The resulting complex increases oxide sorption capacity (Ghanem and Mikkelsen, 1988), but also reduces phosphorus availability and metal exposure (Ivorra et al., 2002).

d) Sorbent composition. Iron-rich suspended solids (e.g. ochre flocs) constitute a significant sink for heavy metals, regulating their partitioning between the sediment and water column (Parkman et al., 1996, Jain and Ram, 1997, Smith,

1999). River bed sediments are thought to play a lesser role in controlling dissolved metal concentrations when suspended sediments are present (Smith, 1999).

e) Concentration of surface-binding sites in the sorbent. When the sorbent becomes limiting, which is common with suspended solids in mine drainage, heavy metals compete with major cations for binding sites (Smith, 1999). In this situation, metals of lower sorption capacity (such as cadmium, nickel and zinc) are often found in the mobile phase (Jain and Ram, 1997, Smith, 1999).

3.1.3 Aims and objectives

The purpose of this chapter is to characterise the chemical processes involved in mine drainage formation and its pollution effects, providing a background upon which the rest of the ecological study on mine drainage pollution is based.

Ochre deposition is thought to be the key pollutant in coal mine drainage, impacting upon SS and iron concentration in water and river bed sediments. Ochre has been observed to cover the stream bed at the study sites (Plates 2.1-2.4, Chapter 2), indicating partial, (pH>5) neutralisation when the drainage reaches the stream (Langmuir, 1997).

Metal mine drainage at the study sites is expected to be characterised by elevated heavy metal concentrations; potentially zinc from sphalerite, lead from galena, iron from pyrite and marcasite and copper and iron from chalcopyrite, as these minerals are known to have been worked in the study mines (Chapter 2) (Bick 1974, Bick 1975, Bick 1977). Lead and

copper are expected to be in higher concentrations in sediments than in water, due to their high sorption affinity onto sediments, whereas zinc concentrations are expected to be higher in water than bed sediments due to its limited sorption capacity when in competition with other cations (Jean and Bancroft, 1986, Sidle et al., 1991, Jain and Ram, 1997, Smith, 1999).

The following chapter analyses the main hydrochemical changes suffered in the study streams due to mine pollution, focusing on the differences between coal and metal mines. These changes probably drive ecological responses in the receiving water courses, hence it is important to understand the chemical processes occurring in these sites *a priori.* The main objectives are:

1. Identify the key differences in water chemistry between coal and metal mine drainage;

2. Establish whether metals are predominantly found in the water or sediment phase within different impacted streams;

3. Determine the environmental variables (water and sediments) affected by mine drainage pollution which will then be used within the ecological study.

3.2 Methodology

Hydrochemical data consisting of water physicochemistry and sediment chemistry were recorded in eight Welsh streams, upstream and downstream from abandoned mine water discharges. Four of the sites were impacted by coal mine drainage and the other four by metal mine drainage (Chapter 2 for details on sampling strategy). The analysis is based on upstream-downstream comparisons. Seasonal variability of stream hydrochemistry is beyond the scope of the present study, thus this aspect is not investigated.

As discussed in the Methodology Chapter (Section 2.1.1), rainfall in the study area was higher during the duration of this study. Streams were sampled in August 2006 (summer), November 2006 (autumn), February 2007 (winter) and May 2007 (spring), in all these months rainfall was higher than average (Table 3.5). Hydrological data is not included in the analysis for this study, however it must be acknowledged that the dataset may not be representative of 'normal' conditions due to weather characteristics during the study.

Table 3.5 Comparison between rainfall averages (1971-2000, Mett Office) and rainfall measurements during the sampling months (2006-2007) in the study areas (data from the Mett Office, 2009)

	Mid Wales		**South Wales**	
Month	2006-2007 (Cwm Ystwyth station)	1971-2000 average (Trawscoed station)	2006-2007 (Cardiff station)	1971-2000 average (Cardiff station)
August	170.2mm	91.2mm	65.6mm	89.9mm
November	228.9mm	140.1mm	178.1mm	117.2mm
February	165.2mm	92.2mm	152.3mm	91.0mm
May	192.5mm	66.0mm	128.5mm	65.0mm

3.2.1 Characterisation of stream chemistry

Dissolved major ions (chloride, nitrate, sulphate, phosphate, calcium, potassium, magnesium and sodium), trace metals in water and sediments (iron, manganese, zinc, copper, cadmium, nickel, lead and chromium), pH, conductivity (EC), total dissolved solids (TDS), suspended solids (SS), settling mineral matter (ash free dry mass, AFDM), temperature, dissolved oxygen (DO), and stream water velocity (to calculate stream discharge) were measured upstream and downstream in the selected study sites of four coal and four metal mines (sampling methods and sites described in Chapter 2).

3.2.2 Ash Free Dry Mass analysis

Ash free dry mass (AFDM) is traditionally used to determine organic matter in biofilms, linking it to algal biomass (Benfield, 1996). Nevertheless, the same method provides information on biofilm mineral content. For this study, mineral matter measurements obtained by AFDM have been used to approximate oxide precipitation onto the river bed.

In summer 2007, six tiles were submerged at each river sampling point and left for 15 weeks to be colonised by algae. At the end of this period, tiles were removed from the water and the biofilm was brushed into a vial. The slurry collected (24 ml) was kept on ice in the dark until frozen in the laboratory.

10 ml of the slurry was oven dried at 80ºC on previously weighed crucibles. The samples were subsequently weighed to obtain dry matter composition. Combustion in a muffle furnace at 550ºC for 2 hours enabled calculation of AFDM. The organic matter is the

fraction combusted in the furnace, whereas the mineral material remains in the crucible (Equation 3.8).

Mineral matter = AFDM – crucible weight (3.8)

3.2.3 PHREEQC

PHREEQC is a computer model designed to perform a wide variety of aqueous geochemical calculations on natural or polluted water. The program is based on an ion-association model and is used here to calculate saturation indices (SI) in the collected samples. The following data were used in the model: dissolved (filtered through 0.2µm) concentrations of Cl^-, NO^{3-}, SO_4^{2-}, PO_4^{3-}, Ca^{2+}, Mg^{2+}, K^+, Na^+, Fe^{3+}, Mn^{2+}, Zn^{2+} and Pb^{2+}; pH, EC, alkalinity and temperature. Alkalinity was calculated using the difference method; the difference in meq of cations and anions was attributed to bicarbonate (Wadham et al., 1998) .

A mineral SI represents the equilibrium condition of a solution with respect to that mineral (Deutsh, 1997). If the SI of a mineral is equal to zero, this means it is in equilibrium with the solution. If the SI is positive, the mineral is oversaturated; and if the SI is negative, the mineral is undersaturated. Negative SI represent the capacity of the water to dissolve the considered mineral, but this can only occur if the mineral is in contact with the water. Exhaustive petrologic data of the study sites is not available to prove the occurrence of dissolution processes, therefore negative SI have not been considered in the present study.

3.2.4 Statistical analysis

Wilcoxon signed rank test (Chapter 2) was used to identify upstream and downstream differences in the stream water and sediment chemistry. A paired method was chosen to overcome the differences between sites and identify the impact caused by the mines. A 5% significance level ($p<0.05$) was used for rejection of the null hypothesis.

Differences in mineral matter deposited (AFDM method) upstream and downstream of the mines were tested using one way ANOVA (Chapter 2). Six standardised mineral matter readings were available per site, and this test was selected to allow comparison of the two populations.

Principal Component Analysis (PCA) was undertaken for hydrochemical data to identify the underlying structure of the data set. This method calculates ordination axes (PCs) that best predict species distribution. PCs were used to determine the major controls upon stream chemistry where mine drainage discharges into the receiving streams by examining correlation indices with measured environmental variables (Leps and Smilauer, 2007).

3.3 Results

3.3.1 Water and sediment chemistry in coal mine impacted streams

Table 3.6 summarises the hydrochemistry of the study sites, the complete set of data can be found in Appendix A. Sulphate concentration significantly ($z=-2.689$, $p=0.007$) increases downstream from coal mine drainage discharge, varying from concentrations of about 16ppm upstream to 118ppm downstream; the specific concentrations vary depending on the stream. Iron ($z=-3.109$, $p=0.002$) and manganese ($z=-2.936$, $p=0.003$) also increase significantly downstream from the mines. Iron concentration range is from 102ppb upstream to 772ppb downstream, whereas manganese is found in lower concentrations, from 12ppb to 700ppb. Zinc is found in very low concentrations (<5ppb) in streams impacted by coal mine drainage and does not significantly ($z=-1.841$, $p>0.05$) vary downstream from the mine discharge. Other trace metals measured in this study include cadmium, copper, lead, nickel and chromium, but they were all below detection limits.

Stream water pH is always around 7, but it significantly ($z=-2.690$, $p=0.007$) decreases downstream from the mine discharge. Similarly, total dissolved solids (TDS) ($z=-3.309$, $p=0.001$) and suspended sediments (SS) ($z=-3.408$, $p=0.001$) increase at the downstream sites, from less than 1ppm SS up to 8ppm SS, and from around 57ppm of TDS to 258ppm. Electrical conductivity (EC) also increases significantly ($z=-3.309$, $p=0.001$) downstream from coal mine discharge, within a range of 84-400µS/cm. Dissolved oxygen

(DO) is always found around saturation levels (100%), but significantly decreases (z=-2.379, p=0.017) downstream from coal mine drainage discharge at the study sites. Stream water discharge does not significantly vary (z=-1.647, p>0.05) between upstream and downstream sites, although specific values vary depending on the stream, ranging from about 5 l/s to 28 l/s.

Iron concentration in sediments significantly (z=-3.059, p=0.002) increases downstream from coal mine discharge at the study sites, from concentrations of about 30000mg/kg to 160000mg/kg. Manganese is found in lower concentrations in the sediments (2000-7000mg/kg), and do not significantly vary (z=-0.549, p>0.05). Similarly to stream water, other metals measured in the sediments (nickel, copper, zinc, lead and cadmium) were below detection limit.

Correlation coefficients between all the environmental variables measured at the coal mine impacted sites are displayed in Table 3.7; those coefficients greater than ±0.5 are marked in bold. Correlation coefficients between conductivity, most ions (SO_4^{2-}, Na^+, Mg^{2+}, K^+ and Ca^{2+}) and TDS are high as all measure the same variable: dissolved ions in the water. SO_4^{2-} is correlated to Fe^{3+} (r=0.53), Mg^{2+} (r=0.87), K^+ (r=0.60) and Ca^{2+} (r=0.85). Mobile Fe^{3+} and Mn^{2+} concentrations in stream water are highly correlated (r=0.79).

Table 3.6 Water chemistry (annual average ± SD, n=4) of streams impacted by coal mine drainage, measured upstream (U/S) and downstream (D/S) of the mine discharge seasonally between summer 2006 and spring 2007

	Aberbeiden		**NantyFyllon**		**Clyne**		**Cwm Gross**	
	U/S	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**
Chloride (ppm)	22.33±9.00	18.02±2.23	11.20±3.21	27.18±34.5	20.29±0.96	19.38±0.90	7.91±1.17	22.61±29.27
Nitrate (ppm)	4.21±2.74	3.75±2.35	2.30±1.83	1.78±1.84	3.78±3.75	3.11±2.63	0.97±0.90	1.14±0.93
Sulphate (ppm)	24.87±16.59	118.09±106.69*	15.77±7.61	42.27±20.72*	72.66±31.42	68.70±36.11*	18.45±20.64	64.38±29.55*
Phosphate (ppm)	0.08±0.05	0.11±0.10	0.15±0.18	0.13±0.10	0.18±0.12	0.10±0.07	0.29±0.310	0.20±0.15
Sodium (ppm)	8.29±0.98	17.36±5.57*	5.70±0.54	5.92±0.46*	11.25±0.97	10.30±1.25*	4.89±0.67	50.74±33.39*
Magnesium (ppm)	3.18±0.70	14.49±7.85*	3.93±1.35	7.05±2.55*	12.95±4.01	12.26±3.97*	1.80±0.25	7.35±3.84*
Potassium (ppm)	1.24±0.09	3.44±1.25*	2.15±0.52	2.93±1.53*	4.84±0.62	3.84±0.62*	0.64±0.07	6.28±3.91*
Calcium (ppm)	7.72±2.94	32.25±25.48*	5.14±2.13	13.42±9.10*	40.36±18.07	38.58±19.08*	5.80±1.97	14.11±9.92*
Iron (ppb)	102.5±56.2	765.0±336.89*	107.5±171.5	353.33±242.15	190.00±209.44*	207.50±249.45	87.50±71.82*	772.5±768.4
Manganese (ppb)	25.0±33.2	695.0±731.3*	35.0±57.5	76.7±86.2*	60.0±120.0	157.4±177.6*	12.5±15.0	107.5±75.9*
Zinc (ppb)	0	5.0±5.8	2.0±4.0	2.7±4.6	BD	4.8±9.6	1.3±2.5	2.3±4.5

*variable significantly ($p<0.05$) changes downstream from the mine discharge according to Wilcoxon signed rank test

Table 3.6 Continuation Sediment and water hydrochemistry (annual average ± SD, n=4) of streams impacted by coal mine drainage, measured upstream (U/S) and downstream (D/S) of the mine discharge between summer 2006 and spring 2007

	Aberbeiden		**NantyFyllon**		**Clyne**		**Cwm Gross**	
	U/S	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**
Iron in sediments (mg/kg)	51010±21910	159790±172000*	29510±4010	37940±49020*	54530±18540	57300±74150*	56650±10270	45730±39610*
Manganese in sediments (mg/kg)	5730±1230	6230±2420	5370±2420	6850±11040	3590±1420	7030±5290	1890±540	2230±1460
pH	7.26±0.15	7.07±0.08*	7.19±0.57	6.92±0.40*	7.40±0.23	7.11±0.12*	6.72±0.47	6.77±0.20*
TDS (ppm)	91.24±16.60	280.50±132.01*	62.19±7.25	120.54±42.93*	257.23±72.89	258.43±69.17*	56.74±11.79	246.63±137.25*
EC (µS/cm)	134.60±24.26	401.40±185.03*	90.70±12.18	176.70±62.26*	368.78±103.35	370.35±97.93*	83.58±18.26	353.33±194.77*
SS (ppm)	3.18±2.60	7.35±3.27*	0.54±0.95	2.44±1.30*	4.74±3.58	6.71±4.41*	3.61±1.07	7.69±2.93*
DO (%)	101±3	103±4*	107±9	105±12*	96±4	92±6*	95±4	79±8*
Discharge (l/s)	16.4±11.6	21.2±12.5	20.11±33.7	26.0±46.6	5.6±6.8	7.5±6.8	33.0±35.9	28.0±26.0
Temperature (ºC)	11.4±1.8	11.3±1.6	11.0±2.3	10.8±2.0	11.1±2.0	11.2±1.8	10.0±5.4	10.7±4.1

*variable significantly ($p<0.05$) changes downstream from the mine discharge according to Wilcoxon signed rank test

Table 3.7 Correlation indices between environmental variables measured in streams impacted by coal mine drainage

	Cl^-	NO_3^-	SO_4^{2-}	PO_4^{3-}	Mn^{2+}	Fe^{3+}	Zn^{2+}	Na^+	Mg^{2+}	K^+	Ca^{2+}	Mn_Sed	Ni_Sed	Fe_Sed	Cu_Sed
Cl^-	1.00	0.16	0.19	0.05	-0.16	0.27	-0.11	**0.51**	0.13	0.46	0.08	-0.29	0.28	0.02	0.04
NO_3^-	0.16	1.00	-0.15	-0.44	0.05	-0.02	0.37	-0.05	0.06	0.01	0.05	0.06	-0.03	0.14	0.07
SO_4^{2-}	0.19	-0.14	1.00	0.14	0.27	**0.53**	-0.08	0.39	**0.87**	**0.60**	0.85	-0.09	-0.24	0.46	-0.18
PO_4^{3-}	0.06	-0.44	0.14	1.00	-0.03	0.13	-0.30	0.21	-0.10	0.11	-0.06	-0.45	-0.05	-0.07	-0.10
Mn^{2+}	-0.16	0.05	0.27	-0.03	1.00	**0.79**	0.40	0.04	0.29	0.09	0.08	0.10	-0.08	**0.60**	0.19
Fe^{3+}	0.27	-0.02	**0.53**	0.13	**0.79**	1.00	0.17	0.27	0.49	0.34	0.31	-0.06	0.13	**0.62**	0.21
Zn^{2+}	-0.11	0.37	-0.08	-0.30	0.40	0.17	1.00	-0.07	0.05	-0.08	-0.05	0.49	-0.11	0.17	0.00
Na^+	**0.51**	-0.05	0.39	0.21	0.04	0.27	-0.07	1.00	0.32	**0.76**	0.21	-0.19	-0.17	0.19	-0.11
Mg^{2+}	0.13	0.06	**0.87**	-0.10	0.29	0.49	0.05	0.32	1.00	**0.68**	**0.95**	0.10	-0.29	0.46	-0.19
K^+	0.46	0.01	**0.60**	0.11	0.09	0.34	-0.08	**0.76**	**0.68**	1.00	**0.64**	-0.14	-0.24	0.23	-0.25
Ca^{2+}	0.08	0.05	**0.85**	-0.06	0.08	0.31	-0.05	0.21	**0.95**	**0.64**	1.00	0.03	-0.33	0.34	-0.29
Mn_Sed	-0.29	0.06	-0.09	-0.45	0.10	-0.06	0.49	-0.19	0.10	-0.14	0.03	1.00	-0.08	-0.09	0.09
Ni_Sed	0.28	-0.03	-0.24	-0.05	-0.08	0.13	-0.11	-0.17	-0.29	-0.24	-0.33	-0.08	1.00	-0.31	**0.52**
Fe_Sed	0.02	0.14	0.46	-0.07	**0.60**	**0.62**	0.17	0.19	0.46	0.23	0.34	-0.09	-0.31	1.00	0.00
Cu_Sed	0.04	0.07	-0.18	-0.10	0.19	0.21	0.00	-0.11	-0.19	-0.25	-0.29	0.09	**0.52**	0.00	1.00
Zn_Sed	0.18	-0.17	**0.73**	-0.06	0.17	0.36	-0.05	0.14	**0.59**	0.14	0.48	0.07	0.03	0.37	0.14
Pb_sed	**0.68**	0.08	-0.06	0.00	-0.06	0.28	-0.09	-0.13	-0.08	-0.08	-0.14	-0.24	0.45	0.00	0.25
pH	-0.29	0.14	-0.13	**-0.57**	0.10	-0.12	0.24	-0.21	0.00	-0.16	0.00	0.37	0.05	-0.14	0.29
EC	0.30	0.03	**0.86**	-0.01	0.20	0.47	0.00	**0.58**	0.93	**0.84**	**0.90**	0.00	-0.34	0.41	-0.26
DO	-0.35	-0.25	-0.09	-0.13	0.19	-0.02	-0.18	**-0.54**	0.01	-0.38	-0.06	0.16	-0.01	0.04	0.08
Temp	-0.20	-0.32	0.08	-0.33	0.26	0.06	-0.08	-0.23	0.17	-0.10	0.11	0.15	-0.18	0.02	0.08
TDS	0.29	0.03	**0.87**	-0.01	0.20	0.47	0.01	**0.58**	**0.93**	**0.84**	**0.90**	0.00	-0.34	0.41	-0.26
Qt	-0.21	-0.03	-0.24	-0.33	-0.02	-0.12	0.38	-0.08	-0.23	-0.29	-0.30	**0.64**	0.07	-0.18	0.19
SS	-0.02	0.00	**0.51**	0.03	**0.51**	0.43	0.21	0.43	0.48	0.48	0.39	0.02	-0.30	0.28	0.08

Table 3.7 Continuation Correlation indices between environmental variables measured in streams impacted by coal mine drainage

	Zn_Sed	Pb_sed	ph	EC	DO	Temp	TDS	Flow	SS
Cl^-	0.18	**0.68**	-0.29	0.29	-0.35	-0.20	0.29	-0.21	-0.02
NO_3^-	-0.17	0.08	0.14	0.03	-0.25	-0.32	0.03	-0.03	0.00
SO_4^{2-}	**0.73**	-0.06	-0.13	**0.86**	-0.09	0.08	**0.87**	-0.24	**0.51**
PO_4^{3-}	-0.06	0.00	**-0.57**	-0.01	-0.13	-0.33	-0.01	-0.33	0.03
Mn^{2+}	0.17	-0.06	0.10	0.20	0.19	0.26	0.20	-0.02	**0.51**
Fe^{3+}	0.36	0.28	-0.11	**0.47**	-0.02	0.06	0.47	-0.12	0.43
Zn^{2+}	-0.05	-0.09	0.24	0.00	-0.18	-0.08	0.01	0.38	0.21
Na^+	0.14	-0.13	-0.21	**0.58**	**-0.54**	-0.23	**0.58**	-0.08	0.43
Mg^{2+}	**0.59**	-0.08	0.00	**0.93**	0.01	0.17	**0.93**	-0.23	0.48
K^+	0.14	-0.08	-0.16	**0.84**	-0.38	-0.10	**0.84**	-0.29	0.48
Ca^{2+}	0.48	-0.14	0.00	**0.90**	-0.06	0.11	**0.90**	-0.30	0.39
Mn_Sed	0.07	-0.24	0.37	0.00	0.16	0.15	0.00	**0.64**	0.02
Ni_Sed	0.03	0.45	0.05	-0.34	-0.01	-0.18	-0.34	0.07	-0.30
Fe_Sed	0.37	0.00	-0.14	0.41	0.04	0.02	0.41	-0.18	0.28
Cu_Sed	0.14	0.25	0.29	-0.26	0.08	0.08	-0.26	0.19	0.08
Zn_Sed	1.00	0.21	-0.01	0.46	0.22	0.19	0.46	0.04	0.18
Pb_sed	0.21	1.00	-0.21	-0.13	0.03	0.00	-0.14	-0.15	-0.24
pH	-0.01	-0.21	1.00	-0.08	0.00	0.33	-0.08	**0.52**	0.03
EC	0.46	-0.13	-0.08	1.00	-0.23	0.04	**1.00**	-0.25	**0.53**
DO	0.22	0.03	0.00	-0.23	1.00	**0.61**	-0.23	-0.15	-0.30
Temp	0.19	0.00	0.33	0.04	**0.61**	1.00	0.03	-0.13	0.21
TDS	0.46	-0.14	-0.08	**1.00**	-0.23	0.03	1.00	-0.24	**0.53**
Qt	0.04	-0.15	**0.52**	-0.25	-0.15	-0.13	-0.24	1.00	-0.07
SS	0.18	-0.24	0.03	**0.53**	-0.30	0.21	**0.53**	-0.07	1.00

PCA produced two components for coal mine impacted streams accounting for 92.5% of the variance in the data. PC 1 was strongly loaded with dissolved Fe^{3+} and Mn^{2+}, and Fe^{3+} in sediment. PC 2 was mainly loaded by Ca^{2+}, TDS, conductivity, K^{+}, Mg^{2+} and SO_4^{2-} (Table 3.8; Figure 3.1). Hence, the hydrochemistry of coal mine impacted streams is dominated by (a) iron and manganese chemistry, and (b) major ion chemistry.

Table 3.8 PCA results for hydrochemical data from coal mine drainage impacted streams between summer2006 and spring 2007

	PC1	**PC2**
EIG	0.7378	0.1869
Cumulative % variance	73.8	92.5
Chloride	0.2253	0.2582
Nitrate	0.0008	0.0094
Sulphate	0.6255	**0.6331**
Phosphate	0.0912	-0.0333
Mn	**0.8097**	-0.401
Fe	**0.9833**	-0.1231
Zn	0.1937	-0.1938
Na	0.3228	0.4786
Mg	0.6077	**0.6944**
K	0.4372	**0.7051**
Ca	0.429	**0.8007**
Mn Sed	-0.0356	-0.0231
Ni Sed	0.0026	-0.3684
Fe Sed	**0.6746**	0.0106
Cu Sed	0.1363	-0.4043
Zn Sed	0.4021	0.3146
Pb sed	0.1695	-0.2117
pH	-0.091	-0.0822
EC	0.5901	**0.8012**
DO	-0.027	-0.2875
Temp	0.0974	-0.0694
TDS	0.5889	**0.8014**
Flow	-0.1488	-0.2113
SS	0.5195	0.2306
Ni	0.6261	-0.3858
Cd Sed	-0.0544	-0.0264

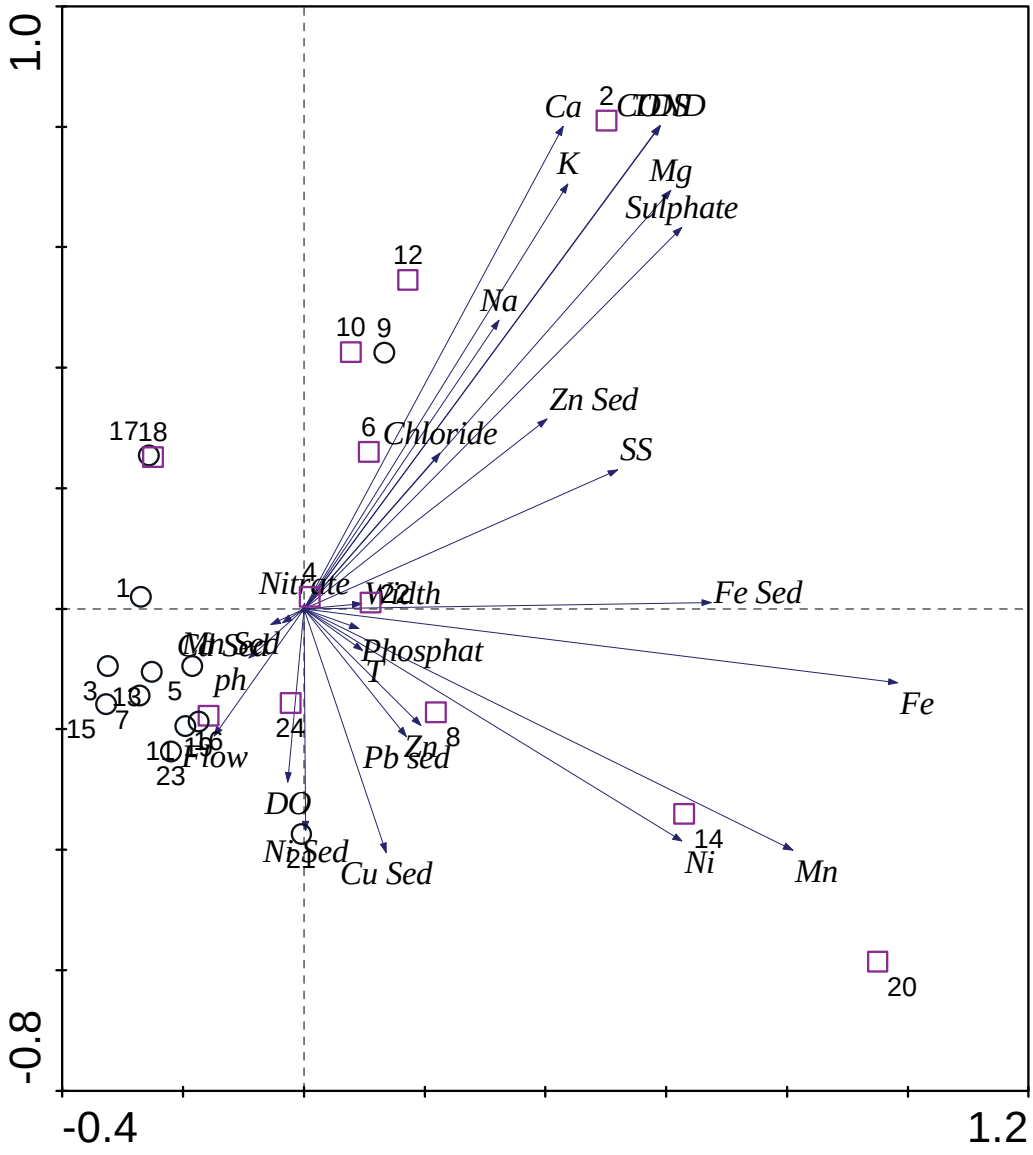


Figure 3.1 Distribution along PCs of upstream (circles) and downstream (squares) samples collected in streams impacted by coal mine drainage between summer 2006 and spring 2007

Figure 3.1 depicts the distribution of samples taken from coal mine impacted streams along PCs. Squares represent downstream samples and circles upstream ones, the arrows show the direction in which the environmental variables increase. Downstream sites tend to group on the right hand site of the graph, where contamination is higher.

Saturation indices were calculated for all elements encountered in the water samples, but only iron oxides presented positive SI values (oversaturation status) (Table 3.9). SI for ochre, goethite and hematite increase downstream from the mine discharge in all sites ($z=-2.845$, $p=0.004$) but Clyne (Table 3.9).

AFDM technique was used to estimate mineral matter being deposited on the stream bed. Figure 3.2 demonstrates an increase of mineral matter ($F=33.878$, $p<0.001$) downstream from coal mine discharge.

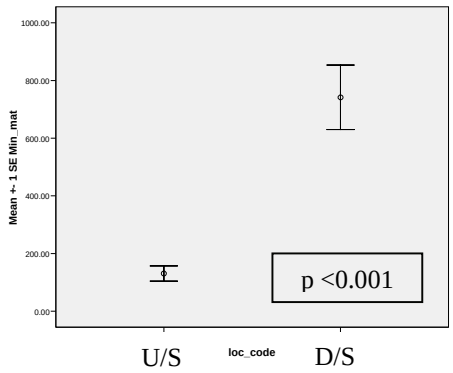


Figure 3.2 Mineral matter (mean ± 1SE, n=48) in biofilms collected upstream (U/S) and downstream (D/S) of coal mine discharge in four impacted streams between summer 2006 and spring 2007

Table 3.9 Upstream and downstream iron compounds saturation indices in sites impacted by coal mine drainage (n=4)

		Upstream				**Downstream**			
		Summer 06	Autumn 06	Winter 07	Spring 07	Summer 06	Autumn 06	Winter 07	Spring 07
Clyne	Ochre	1.71	2.23	0	2.32	0	1.3	0	1.81
	Goethite	7.17	7.52	0	7.74	0	6.6	0	7.21
	Hematite	16.28	16.97	0	17.43	0	12.38	0	16.36
Cwm Gros	Ochre	0	-1.9	-0.19	1.56	1.53	0.05	0.34	1.44
	Goethite	0	3.2	5.02	6.96	7.07	5.27	5.57	6.87
	Hematite	0	8.31	11.96	15.87	16.11	12.45	13.05	15.68
NantyFyllon	Ochre	0	-0.1	0	2.22	0.14	0.54	2.4	
	Goethite	0	5.21	0	7.58	5.58	5.9	7.71	
	Hematite	0	12.36	0	17.09	13.1	13.74	17.34	
Aberbaiden	Ochre	1.35	1.2	1.41	2.24	1.9	1.42	1.73	2.42
	Goethite	6.83	6.53	6.74	7.64	7.36	6.75	7.06	7.82
	Hematite	15.61	15	15.41	17.21	16.67	15.43	16.06	17.58

Table 3.10 Wilcoxon rank test results comparing ochre, goethite and hematite saturation indices upstream and downstream from coal mine drainage discharge (Clyne is not included)

	Ochre	Goethite	Hematite
p values	0.004	0.004	0.004

3.3.2 Water and sediment chemistry in metal mine impacted streams

Table 3.11 displays annual means for hydrochemical variables measured in four streams impacted by metal mine drainage in Wales. Water chemistry measurements indicate a significant increase in SO_4^{2-} from 2.82ppm to 10ppm (z=-3.464, p=0.01), TDS from 31.71ppm to 69.22ppm (z=-2.223, p=0.023), conductivity from 46.12µS/cm to 109.76 µS/cm (z=-2.964, p=0.003), Zn^{2+} from 6.25ppb to 1450ppb (z=-2.760, p=0.006), Cl^- from 5.99ppm to 12.40ppm (z=-2.172, p=0.03) and NO_3^- from 0.82ppm to 3.77ppm (z=-2.482, p=0.013) downstream from the mine discharge. Other major ions such as PO_4^{3-} (<1ppm) (z=-0.534, p>0.05), Na^+ (3.98-9.03ppm) (z=-2.017, p>0.05), Mg^{2+} (0.84-2.85ppm) (z=-1.506, p>0.05), K^+ (0.30-0.72) (z=-2.238, p>0.05) and Ca^{2+} (1.30-6.90ppm) (z=-1.506, p>0.05) do not vary significantly between the upstream and downstream sites. Mn^{2+} (2.5-20ppb) (z=-0.378, p>0.05), Fe^{3+} (30-125ppb) (z=-1.892, p>0.05) and Pb^{2+} (from below detection limit up to 157ppb) (z=-1.703, p>0.05) concentrations at the study sites are highly variable, but do not appear to significantly vary downstream from the mine discharge. Cadmium was below detection limit in all the water samples collected. Suspended sediments (<2.09ppm) (z=-1931, p>0.05) and pH (around 7) (Z=-0.973, p>0.05) do not significantly vary downstream from metal mine discharge. Stream flow is highly variable between sites (4-242.6 l/s), but not between upstream and downstream from the mines (z=-0.569, p>0.05).

Metal concentrations in sediments significantly increased downstream from metal mine discharge; Zn^{2+} from 640mg/kg to 6440mg/kg (z=-2.746, p=0.006), Pb^{2+} from 50mg/kg to 62650mg/kg (z=-2.040, p=0.041) and Cu^{2+} from below detection limit to 1460mg/kg (z=-2.476, p=0.013). Fe^{3+} (40500-127500mg/kg) (z=-1.412, p>0.05) and Mn^{2+} (2200-45500mg/kg) (z=-0.392, p>0.05) are also detected in the sediments, but, even though they appear to increase at the downstream sites when considering mean values, there are no significant differences probably due to high standard deviation values. Nickel and cadmium concentrations in sediments were below detection limits.

Correlation coefficients displayed in Table 3.12 indicate high correlation between conductivity, most ions (Cl^-, SO_4^{2-}, Na^+, Mg^{2+}, K^+ and Ca^{2+}) and TDS (r>0.64). Dissolved Mn^{2+} appears to be highly correlated to pH (r=0.77). Pb^{2+}, Zn^{2+} and Cu^{2+} concentrations in sediment appear to be correlated (r>0.66)

Table 3.11 Water chemistry (annual average ± SD, n=4) of streams impacted by metal mine drainage, measured upstream (U/S) and downstream (D/S) of the mine discharge seasonally between summer 2006 and spring 2007 (BD: below detection limit**)**

	Dylife		**Frongoch**		**Cwm Ystwyth**		**Cwm Symlog**	
	U/S	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**
Chloride (ppm)	8.05±2.15	9.64±2.26*	9.47±0.84	9.93±1.49*	5.99±0.69	7.27±2.23*	11.98±2.05	12.40±2.47*
Nitrate (ppm)	3.14±2.35	3.68±1.69*	2.26±1.53	2.55±1.10*	0.82±0.51	1.19±0.47*	1.21±0.47	3.77±1.42*
Sulphate (ppm)	2.82±0.64	8.68±5.05*	6.25±1.43	10.09±3.02*	3.71±0.83	5.79±2.61*	5.50±0.58	10.87±1.17*
Phosphate (ppm)	0.22±0.16	0.23±0.16	0.19±0.15	0.19±0.15	0.22±0.14	0.24±0.16	0.20±0.15	0.21±0.15
Zinc (ppb)	87.50±175.00	942.50±944.72*	35.50±39.85	1450.00±1190.24*	6.25±9.46	410.00±318.43*	22.50±45.00	64.25±123.88*
Manganese (ppb)	10.0±11.6	7.5±9.6	5.0±5.8	2.5±5.0	10.0±20.0	10.0±20.0	5.0±10.0	20.0±33.7
Iron (ppb)	125.0±107.9	32.5±42.7	80.0±57.2	45.0±42.0	67.5±66.2	60.0±60.6	30.0±42.4	45.0±37.0
Lead (ppb)	47.5±55.0	157.5±152.4	BD	42.5±50.6	BD	BD	BD	25.0±50.0
pH	7.09±0.38	6.98±0.21	6.79±0.19	6.67±0.13	7.11±0.41	6.89±0.37	7.02±0.75	7.28±0.86
Flow (l/s)	10.4±10.2	37.0±28.1	4.0±4.2	8.3±7.9	242.6±190.7	117.2±99.1	3.8±4.0	17.1±17.7
SS (ppm)	1.45±1.51	0.97±1.51	1.69±1.64	1.31±1.03	1.70±2.19	0.49±0.56	2.09±2.26	1.28±1.27
EC (µS/cm)	55.87±5.57	74.64±16.33*	86.56±21.05	91.56±19.95*	46.12±11.43	47.25±11.36*	66.36±6.73	109.76±19.18*

*variable significantly ($p<0.05$) changes downstream from the mine discharge according to Wilcoxon signed rank test

Table 3.11Continuation Water chemistry (annual average ± SD, n=4) of streams impacted by metal mine drainage, measured upstream (U/S) and downstream (D/S) of the mine discharge seasonally between summer 2006 and spring 2007 (BD: below detection limit**)**

	Dylife		**Frongoch**		**Cwm Ystwyth**		**Cwm Symlog**	
	U/S	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**
TDS (ppm)	38.33±3.71	51.13±10.91*	59.20±13.69	69.22±14.21*	31.71±8.04	32.46±7.57*	45.32±5.39	59.88±29.76*
Sodium (ppm)	4.97±1.74	4.91±1.59	6.46±1.68	6.42±0.93	3.98±0.53	4.54±0.72	7.19±2.31	9.03±2.98
Magnesium (ppm)	1.25±0.43	1.43±0.64	2.04±0.80	1.79±0.43	0.84±0.12	1.14±0.34	1.56±0.43	2.85±1.43
Potassium (ppm)	0.47±0.25	0.56±0.32	0.51±0.11	0.56±0.10	0.30±0.08	0.34±0.12	0.33±0.06	0.72±0.20
Calcium (ppm)	2.94±0.38	3.77±1.59	6.56±4.04	5.33±1.70	1.30±0.54	2.23±1.82	3.15±0.98	6.90±4.60

*variable significantly ($p<0.05$) changes downstream from the mine discharge according to Wilcoxon signed rank test

Table 3.11 Continuation Sediment chemistry (annual average ± SD, n=4) of streams impacted by metal mine drainage, measured upstream (U/S) and downstream (D/S) of the mine discharge seasonally between summer 2006 and spring 2007 (BD: below detection limit**)**

	Dylife		**Frongoch**		**Cwm Ystwyth**		**Cwm Symlog**	
	U/S	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**	**U/S**	**D/S**
Zinc in sediments (mg/kg)	5510±8020	6440±2460*	2080±1380	4150±1840*	640±640	2620±1930*	860±740	1870±1150*
Lead in sediments (mg/kg)	31710±51680	62650±1.700*	2400±2100	25720±19310*	50±60	7870±5730*	18610±20910	22780±10100*
Copper in sediments (mg/kg)	780±1100	1460±410*	BD	90±80*	BD	80±70*	40±30	930±1260*
Manganese in sediments (mg/kg)	16900±15000	33100±56700	2600±2700	23500±42300	2200±1900	2900±700	4800±6400	45500±86600
Iron in sediments (mg/kg)	64200±15000	127500±63400	70100±19700	75000±19200	86500±76300	50300±2100	40500±23900	80400±36100

*variable significantly ($p<0.05$) changes downstream from the mine discharge according to Wilcoxon signed rank test

Table 3.12 Correlation indices between environmental variables measured in streams impacted by metal mine drainage

	Chloride	**Nitrate**	**Sulphate**	**Phosphate**	**Mn**	**Fe**	**Zn**	**Pb**	**Na**	**Mg**	**K**	**Ca**
Chloride	1.00	0.18	**0.58**	0.18	0.08	-0.45	0.22	0.15	**0.85**	**0.72**	0.43	0.49
Nitrate	0.18	1.00	0.40	-0.49	0.01	0.18	0.03	0.20	0.08	0.12	**0.63**	0.19
Sulphate	**0.58**	0.40	1.00	0.12	-0.12	-0.42	0.63	0.44	0.43	**0.55**	**0.64**	**0.58**
Phosphate	0.18	-0.49	0.12	1.00	0.03	-0.38	0.30	0.17	0.16	0.27	0.15	0.30
Mn	0.08	0.01	-0.12	0.03	1.00	0.02	-0.19	-0.13	0.09	-0.09	-0.10	-0.33
Fe	-0.45	0.18	-0.42	-0.38	0.02	1.00	-0.35	-0.06	-0.36	-0.33	-0.08	-0.29
Zn	0.22	0.03	0.63	0.30	-0.19	-0.35	1.00	**0.51**	0.04	0.15	0.33	0.23
Pb	0.15	0.20	0.44	0.17	-0.13	-0.06	**0.51**	1.00	0.07	0.20	**0.51**	0.14
Na	**0.85**	0.08	0.43	0.16	0.09	-0.36	0.04	0.07	1.00	**0.86**	0.43	**0.55**
Mg	**0.72**	0.12	**0.55**	0.27	-0.09	-0.33	0.15	0.20	**0.86**	1.00	**0.62**	**0.83**
K	0.43	0.63	**0.64**	0.15	-0.10	-0.08	0.33	0.51	0.43	**0.62**	1.00	**0.68**
Ca	0.49	0.19	**0.58**	0.30	-0.33	-0.29	0.23	0.14	0.55	**0.83**	**0.68**	1.00
MnSed	-0.13	0.63	0.07	-0.49	-0.19	0.26	-0.18	0.00	-0.14	-0.12	0.19	0.00
NiSed	-0.02	-0.23	0.07	0.20	-0.26	-0.01	-0.06	-0.19	0.06	0.13	-0.12	0.07
FeSed	-0.17	0.43	0.15	-0.02	0.09	0.00	0.13	0.34	-0.24	-0.10	0.42	0.00
CuSed	0.38	0.28	0.28	0.05	0.17	-0.13	0.18	**0.59**	0.49	**0.48**	0.48	0.16
ZnSed	0.09	0.35	0.06	-0.04	0.09	-0.16	0.30	0.45	0.10	0.10	0.36	-0.01
PbSed	0.24	0.41	0.25	-0.07	0.04	-0.21	0.35	0.61	0.20	0.17	0.46	0.02
ph	0.22	-0.12	-0.11	0.24	0.77	-0.26	-0.19	-0.12	0.18	0.08	-0.13	-0.16
EC	**0.67**	0.37	**0.78**	0.21	-0.04	-0.31	0.34	0.17	**0.64**	**0.78**	**0.76**	**0.85**
Temp	0.34	-0.27	0.31	0.67	-0.39	-0.32	0.21	0.11	0.31	0.36	0.22	0.49
TDS	0.34	0.39	**0.59**	0.09	-0.05	-0.29	0.33	0.10	0.22	0.33	**0.54**	**0.63**
Flow	-0.51	-0.24	-0.37	-0.23	0.20	0.29	-0.21	-0.18	-0.39	-0.40	-0.34	-0.42
SS	-0.16	0.03	-0.22	-0.21	0.19	0.39	-0.15	-0.26	-0.16	-0.17	-0.06	-0.05

Table 3.12 Continuation Correlation indices between environmental variables measured in streams impacted by metal mine drainage

	MnSed	NiSed	FeSed	CuSed	ZnSed	PbSed	ph	COND	T	TDS	Flow	SS
Chloride	-0.13	-0.02	-0.17	0.38	0.09	0.24	0.22	**0.67**	0.34	0.34	**-0.51**	-0.16
Nitrate	**0.63**	-0.23	0.43	0.28	0.35	0.41	-0.12	0.37	-0.27	0.39	-0.24	0.03
Sulphate	0.07	0.07	0.15	0.28	0.06	0.25	-0.11	**0.78**	0.31	**0.59**	-0.37	-0.22
Phosphate	-0.49	0.20	-0.02	0.05	-0.04	-0.07	0.24	0.21	**0.67**	0.09	-0.23	-0.21
Mn	-0.19	-0.26	0.09	0.17	0.09	0.04	**0.77**	-0.04	-0.39	-0.05	0.20	0.19
Fe	0.26	-0.01	0.00	-0.13	-0.16	-0.21	-0.26	-0.31	-0.32	-0.29	0.29	0.39
Zn	-0.17	-0.06	0.13	0.18	0.30	0.35	-0.19	0.34	0.21	0.33	-0.21	-0.15
Pb	0.00	-0.19	0.34	**0.60**	0.45	**0.61**	-0.12	0.17	0.11	0.10	-0.18	-0.26
Na	-0.13	0.05	-0.24	0.49	0.10	0.20	0.18	**0.64**	0.31	0.22	-0.39	-0.16
Mg	-0.12	0.13	-0.10	0.48	0.10	0.17	0.08	**0.78**	0.36	0.33	-0.40	-0.17
K	0.19	-0.12	0.42	0.48	0.36	0.46	-0.13	**0.76**	0.22	**0.54**	-0.34	-0.06
Ca	0.00	0.07	0.00	0.16	-0.01	0.02	-0.16	**0.85**	0.49	**0.63**	-0.42	-0.05
MnSed	1.00	-0.25	0.39	-0.04	0.03	0.07	-0.26	0.04	-0.17	0.16	-0.07	0.16
NiSed	-0.25	1.00	-0.40	-0.02	-0.26	-0.21	-0.15	-0.09	0.12	-0.33	-0.13	-0.14
FeSed	0.39	-0.40	1.00	0.10	0.21	0.24	0.03	0.14	-0.13	0.23	0.11	0.13
CuSed	-0.04	-0.02	0.09	1.00	**0.66**	**0.72**	0.24	0.19	-0.06	-0.20	-0.17	-0.25
ZnSed	0.03	-0.26	0.21	**0.66**	1.00	**0.80**	0.07	0.00	-0.32	0.01	-0.18	-0.20
PbSed	0.07	-0.21	0.24	0.72	**0.80**	1.00	-0.03	0.10	-0.16	0.03	-0.26	-0.08
pH	-0.26	-0.15	0.03	0.24	0.07	-0.03	1.00	-0.01	-0.16	-0.09	-0.02	-0.05
EC	0.04	-0.09	0.14	0.19	0.00	0.10	-0.01	1.00	0.41	**0.78**	-0.44	0.04
Temp	-0.17	0.12	-0.13	-0.06	-0.32	-0.16	-0.16	0.40	1.00	0.28	-0.38	-0.24
TDS	0.16	-0.33	0.23	-0.20	0.01	0.03	-0.09	**0.78**	0.28	1.00	-0.39	0.09
Flow	-0.07	-0.13	0.10	-0.17	-0.18	-0.26	-0.02	-0.44	-0.38	-0.39	1.00	0.20
SS	0.16	-0.14	0.13	-0.25	-0.20	-0.08	-0.05	0.04	-0.24	0.09	0.20	1.00

PCA produced one component for metal mine impacted streams accounting for 95.9% of variance in the data. PC1 is strongly loaded with dissolved Zn^{2+} and SO_4^{2-} (Table 3.13). Figure 3.4 depicts the distribution of samples collected in metal mine impacted streams along PCs. Once more, squares represent downstream sites, whereas circles represent upstream sites. Similarly to the case of coal mine impacted sites, downstream samples tend to gather on the right hand side of the graph, where contamination is higher.

Table 3.13 PCA results for coal and metal mine hydrochemistry data between summer 2006 and spring 2007

	PC1
EIG	0.9588
Chloride	0.2266
Nitrate	0.0269
Sulphate	**0.6294**
Phosphate	0.3031
Mn	-0.1868
Fe	-0.3563
Zn	**1**
Cd	0.3481
Pb	0.5166
Na	0.0474
Mg	0.1486
K	0.3348
Ca	0.2318
MnSed	-0.1744
NiSed	-0.0641
FeSed	0.1344
CuSed	0.1847
ZnSed	0.3029
PbSed	0.3548
CdSed	-0.125
pH	-0.1896
EC	0.3439
Temp	0.2164
TDS	0.3271
Flow	-0.211
SS	-0.157

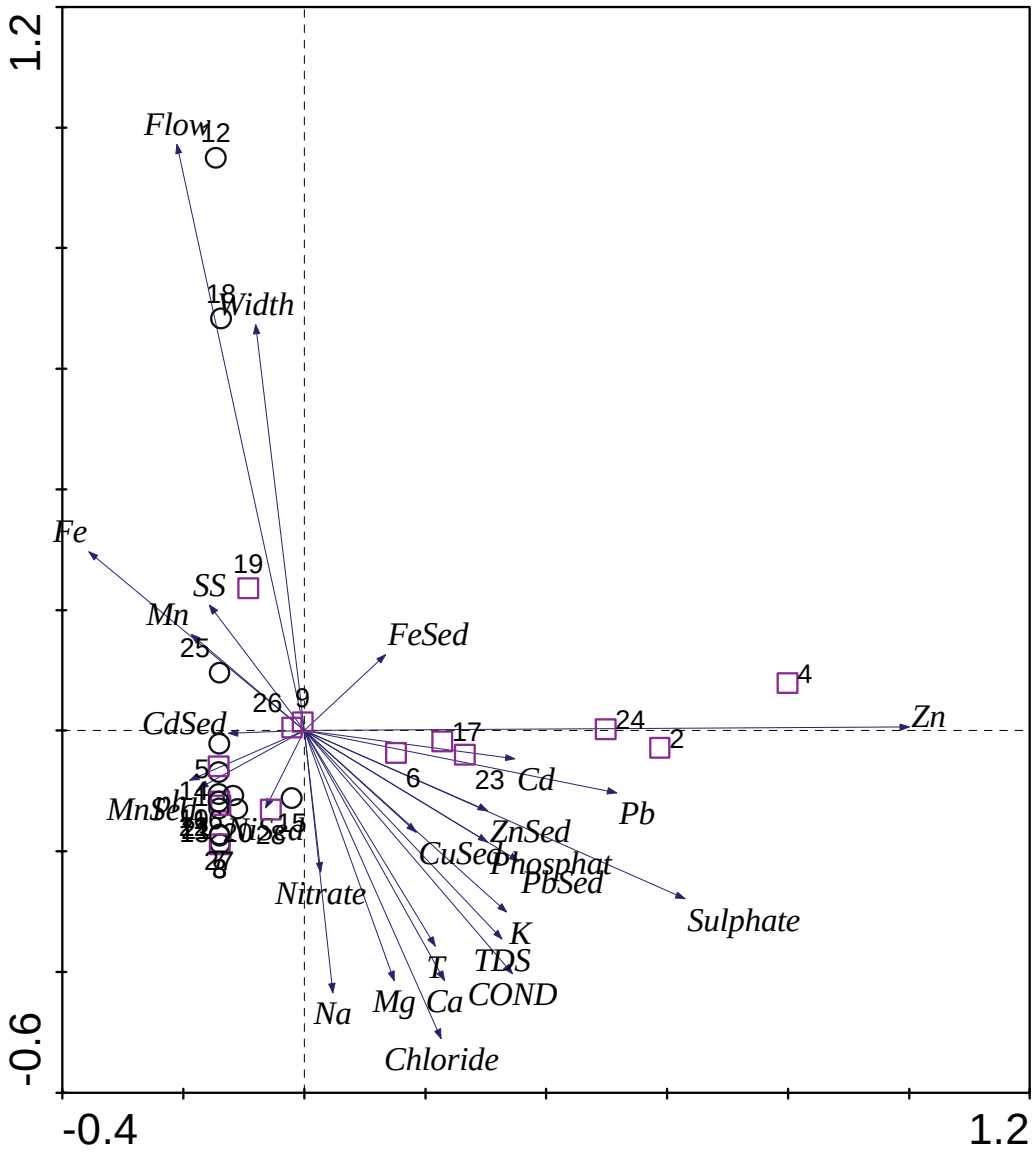


Figure 3.3 Distribution along PCs of upstream (circles) and downstream (squares) samples collected in four streams polluted by metal mine drainage between summer 2006 and spring 2007

Saturation indices (SI) were calculated for all elements in solution at streams impacted by metal mine drainage. Only iron compounds presented positive values, and these are displayed in Table 3.14. There are no significant differences between SI calculated upstream and downstream from the metal mines for ochre ($z=-1.490$, $p>0.05$), goethite ($z=-1.255$, $p>0.05$) or hematite ($z=-1.256$, $p>0.05$), however, in most cases they are positive.

Data obtained from AFDM measurements suggest there is no significant change in mineral matter deposition upstream and downstream from metal mine drainage discharge ($F=0.089$, $p=0.767$) (Figure 3.4).

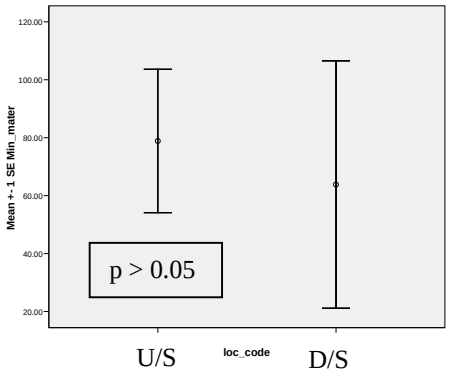


Figure 3.4 Mineral matter (mean ± 1SE, n=48) in biofilms collected upstream (U/S) and downstream (D/S) of metal mine discharge in four impacted streams between summer 2006 and spring 2007

Table 3.14 Upstream and downstream iron compounds saturation indices in sites impacted by metal mine drainage (n=4)

		Upstream				Downstream			
		Summer 06	Autumn 06	Winter 07	Spring 07	Summer 06	Autumn 06	Winter 07	Spring 07
Cwm Symlog	Ochre	0	-1.27	2.42	0	0	-0.75	2.38	1.25
	Geothite	0	3.96	7.64	0	0	4.51	7.65	6.68
	Hemathite	0	9.84	17.2	0	0	10.94	17.23	15.31
Cwm Ystwyth	Ochre	0	-0.46	1.92	0.89	0	-0.54	1.36	-0.92
	Geothite	0	4.77	7.11	6.16	0	4.69	6.55	4.34
	Hemathite	0	11.46	16.13	14.24	0	11.29	15.02	10.61
Dylife	Ochre	0	0.42	1.46	2.01	0	0.67	0.93	0
	Geothite	0	5.61	6.6	7.41	0	5.89	6.1	0
	Hemathite	0	13.13	15.11	16.76	0	13.7	14.12	0
Frongoch	Ochre	0	-0.53	0.45	0.42	0	-0.63	-0.05	-0.44
	Geothite	0	4.75	5.69	5.72	0	4.66	5.22	4.9
	Hemathite	0	11.42	13.31	13.36	0	11.25	12.36	11.74

Table 3.15 Wilcoxon rank test comparing ochre, goethite and hematite saturation indices upstream and downstream from metal mine drainage discharge

	Ochre	Goethite	Hematite
p values	0.136	0.209	0.209

3.4 Discussion

3.4.1 Coal mine pollution

3.4.1.1 Water and sediment chemistry

Pyrite (FeS_2) is the main mineral found in coal. When pyrite gets in contact with oxygenated waters, sulphates, iron and acidity are released to the environment (Equations 3.1-3.4). The hydrochemical data from coal mine impacted sites forming this study (Table 3.6), show a significant increase in these components downstream from the mine discharge, demonstrating that pyrite weathering is an important process regulating the chemistry of these streams.

Mn^{2+} is often associated with coal mine drainage pollution (Table 3.1), however its origin has no easy interpretation, as minerals containing manganese such as rhodochrosite ($MnCO_3$) and pyrolusite (MnO_2) are not common in coal bearing strata (Larsen and Mann, 2005). Larsen and Mann (2005) identified two main manganese sources (a) from clay minerals forming shale or mudstone, and (b) from siderite ($FeCO_3$) concretions in the shale, mudstone or sandstone. Impure siderite contains a small proportion of Mn^{2+}, Mg^{2+} and Ca^{2+} which substitutes some of the Fe^{3+}. A combination of both sources is thought to occur, the latter being the most important.

Marine and freshwater shale is known to occur in the South Wales coalfields (Moore, 1954), and the main components in the catchment bedrock are mudstone and sandstone (BGS, 1997). Therefore, the presence of shale, mudstone and sandstone in the study catchment, in conjunction with the possible presence of impure siderite and even other minerals such as gypsum ($CaSO_4$), kainite ($MgSO_4 \cdot KCl \cdot H_2O$), schönite ($K_2SO_4 \cdot MgSO_4 \cdot 6H_2O$) and leonite ($K_2SO_4 \cdot MgSO_4 \cdot 4H_2O$) may explain the significant increase of Mn^{2+}, Ca^{2+}, K^+ and Mg^{2+} downstream from the studied coal mine drainage discharges. However, due to the lack of exhaustive petrologic data for the area, the presence of these minerals can not be confirmed.

Most of British coal is known to be “saline”, causing an enrichment of chloride in coal mine drainage (Yudovich and Ketris, 2006). Even though the origins of Cl^- are still uncertain, concentrations in coal have often been correlated with sodium, suggesting a common origin as either NaCl or within the organic fraction in the coal (Yudovich and Ketris, 2006).

In the present study, significant differences in chloride concentrations upstream and downstream from coal mines were not detected. Sodium did significantly increase in the stream after mixing with mine drainage, and presented a correlation index of 0.51 with chloride, which might suggest a common origin as NaCl. However, the data gathered for the present study cannot confirm the hypothesis of Yudovich and Ketris (2006).

PCA summarises the effect of coal mine drainage on the receiving stream hydrochemistry. Variables originated in coal mines (Fe^{3+}, Mn^{2+}, Fe^{3+} in sediments, SO_4^{2-} and major cations) can explain 92.5% of the variation amongst the samples. This can be visualised in the gathering of downstream samples at the right hand side of Figure 3.1, where pollution is higher. Hence, a clear effect of coal mine drainage on water and sediment chemistry has been demonstrated.

The streams impacted by coal mine drainage studied in the present project do not seem to be severely contaminated when compared with others from the literature (Table 3.1). As Table 3.1 and the present study suggest, there is high variability of metal concentrations and acidity between streams impacted by the drainage from different collieries. However, these are often reported to be more acidic (pH as low as 2) than the study streams (pH around 7). They usually contain more iron in solution, with concentrations of 1400ppm reported in River Avoca (Gray, 1998) and 500ppm in Rio Tinto (Canovas et al., 2008), but more commonly 3-7ppm; whereas average iron concentration at the study streams does not exceed the EQS of 1ppm (Table 1.1, Chapter 1). This higher concentration of dissolved iron is probably due to the lower pH found in these rivers, as iron remains in solution in pH<5 (Langmuir, 1997). Manganese concentrations in streams impacted by coal mine drainage are often lower than iron concentrations (up to 25ppm in Rio Tinto (Canovas et al., 2008), but normally 3-4ppm), however they are still higher than in the study sites (up to 650ppb). Nevertheless, in both cases the EQS of 17.5ppb set for manganese is exceeded. Sulphate concentrations at the study sites (up to 106ppm) are within the range of those recorded in the literature (28-400ppm), except heavily polluted

streams such as Rio Tinto (2583ppm) (Canovas et al., 2008). Similarly to manganese, sulphate often exceeds the EQS of 87.5ppm. Metals other than manganese and iron can be associated with coal mine drainage pollution (Table 3.1), but this does not seem to be the case in the study sites, as they were recorded either in very low concentrations (less than 5ppb of zinc) or below detection limits.

3.4.1.2 Water-sediment interphase

There are two main vectors of transport of heavy metals from the water column into the sediments (a) precipitation as oxides and (b) adsorption and sedimentation via suspended sediments. Water and sediment analysis in combination with SI calculation has enabled the interpretation of the fate of heavy metals within the water column.

Hydrochemical data available for streams impacted by coal mine drainage suggest that downstream from coal mines Fe^{3+} is precipitating out of solution as ochre, goethite and hematite; it aggregates to form flocs (SS) and settles on the stream bed, increasing Fe^{3+} concentration and mineral matter content in sediments (AFDM). Oxide precipitation thus appears to be the main vector of Fe^{3+} transfer from the water column into the sediments.

Direct precipitation of manganese oxides and their importance as trace metal scavengers in water polluted by mine drainage has been reported (Balistrieri and Murray, 1986, Fu et al., 1991, Lee et al., 2002, Butler et al., 2008), yet to the author's knowledge adsorption of manganese onto other solids has not been described. In the present study, SI do not indicate precipitation of Mn^{2+} in any form and its concentration in sediments, although considerable, does not increase significantly at the downstream sites. Mn^{2+} is known to

remain mobile in slightly acidic or neutral conditions and has often been found dissolved in acid mine drainage (Kimball et al., 1995). Lee et al. (2002) did not observe Mn^{2+} oxides precipitating before reaching pH of 8. This may suggest that Mn^{2+} precipitates from the water column only when the conditions are favourable (high pH), thus limiting their transfer into the sediments. Such conditions did not occur during stream sampling, as pH never reached a value of 8. Additionally, presence of Fe^{2+} in solution has been seen to dissolve manganese oxides (Villinski et al., 2001) and to prevent their formation (Gouzinis et al., 1998) in previous studies. Therefore, manganese concentration in sediments from streams polluted by coal mine drainage is difficult to predict, as it does not only respond to Mn^{2+} concentration in solution, but also to pH and Fe^{2+} concentration in the water. This might explain the lack of statistically significant variations observed in Mn^{2+} concentration in sediments, despite its increase in the water column.

Contrary to water chemistry, sediment collected at the study sites suggest that these streams are similarly polluted to many found in the literature (Table 3.2). Iron concentrations in sediments are often between 4000-51000mg/kg in the literature, and this is sometimes exceeded at the study sites, with average concentrations of 29510-159790mg/kg. Similarly, manganese is reported at concentrations of 72-27900mg/kg in the literature (Table 3.2) and the values at the study sites (1890-7030mg/kg) are within this range. Metals other than iron and manganese (e.g. zinc, cadmium and lead) have been found in other streams, however in the present study these were below detection limits. There are no mandatory standards in the UK in relation to metal concentrations in sediments (Chapter 1). The EA is currently developing a programme to establish

sediment quality guidelines to facilitate the achievement of environmental objectives set by the Water Framework Directive (Hudson-Edwards et al. 2008) (Chapter 1). However, as the project is focused on metal mine pollution, manganese and iron are not covered in the first draft.

3.4.2 Metal mine pollution

3.4.2.1 Water and sediment pollution

Galena (PbS), sphalerite (ZnS), pyrite (FeS_2), chalcopyrite ($CuFeS_2$) and marcasite (FeS_2) have been reported as present in the studied metal mines (Bick, 1974, Bick, 1975, Bick, 1976, Bick, 1977) (Chapter 2), hence there is a potential for the presence of Pb^{2+}, Zn^{2+}, Fe^{3+} and Cu^{2+} in the contaminated waters. Hydrochemical data from these sites show that sphalerite weathering is the main process occurring in the mines. During sphalerite weathering, SO_4^{2-} and Zn^{2+} are released into the environment (Equation 3.4), and this is reflected in their significant increase in concentration downstream from metal mine discharge.

SO_4^{2-} concentration in the study streams is correlated to Zn^{2+}, but also to other cations (Mg^{2+}, Ca^{2+} and K^{+}), suggesting there may be other minerals in the catchment contributing to its increase in concentration, e.g. gypsum ($CaSO_4$), minerals containing

K_2SO_4 and $MgSO_4$ such as kainite ($MgSO_4 \cdot KCl \cdot H_2O$) schönite ($K_2SO_4 \cdot MgSO_4 \cdot 6H_2O$) and leonite ($K_2SO_4 \cdot MgSO_4 \cdot 4H_2O$). However, exhaustive petrologic data of the area confirming their presence is not available.

Increase in nitrate concentrations (0.82-3.77ppm) observed in metal mine polluted streams is probably due to agricultural inputs (Kim et al., 2007), since grazing is the main land use in this area. The origin of the high chloride concentrations in these streams (5.99-12.40ppm) is uncertain, although it may originate in pyrite traces found in the ore worked in these mines (Bick, 1974) associated with NaCl as described for coal mines.

Sediments collected in these sites show an increase in Pb^{2+}, Zn^{2+} and Cu^{2+} concentrations downstream from the mine drainage. It is important to observe that sediment chemistry is highly influenced by galena (PbS), pyrite (FeS_2) and chalcopyrite ($CuFeS_2$) weathering, as well as sphalerite (ZnS). Section 3.4.2.2 investigates the processes involved in this transport of heavy metals from the water column to the sediment, as well as the absence of Pb^{2+} and Cu^{2+} in water.

PCA summarises the effect of metal mine drainage on stream hydrochemistry. Variables directly linked to sphalerite (ZnS) weathering, i.e. Zn^{2+} and SO_4^{2-} (Equation 3.5), explain 95.9% of the sample variation. Similarly to coal mine impacted streams, downstream samples gather on the right hand side of Figure 3.3, where variables related to metal mine drainage increase in concentration. Thus, the chemical impact of metal mine drainage on stream water and sediment is demonstrated.

Comparing water chemistry at the study sites with examples of streams impacted by metal mine drainage in the literature (Table 3.3), it appears that both are within the same range of metal concentrations and pH. Metal mine drainage polluted streams are normally characterised by high metal concentrations, the specific metals depending on the minerals present in the bedrock. These (Table 3.3) are often zinc (83-1480ppb), lead (0.4-68ppb), copper (5.9-41ppb, with heavily polluted streams such as Estanda in Spain with much higher concentrations of 2570ppb (Marques et al., 2003)), cadmium (from below detection limit to 530ppb) and nickel (around 3ppb). At the study sites (Table 3.11), zinc and lead are within the same range and both often exceed the EQS of 120ppb Zn^{2+} and 4ppb Pb^{2+}. Copper, cadmium and nickel are below detection limit at the study sites..

Due to the reactions involved in weathering of non-ferrous minerals (Equations 3.5-3.7), pH of streams impacted by metal mine drainage is often circumneutral. However, if pyrite is present in the bedrock (as suggested by the presence of iron in the water), pH can reach lower values (4-8). In the study streams, pH values lower than 6.31 were never recorded, therefore is within the EQS of 6-9.

Metal mine impacted streams present similar pH values to coal mine impacted streams studied in this project (around 7), the main differences being in trace metal concentrations measured in the water column. Coal mine polluted streams have higher iron (87.5-765ppb), manganese (12.5-695 ppb) and sulphate (15.77-118.09ppm) concentrations than metal mine polluted streams (30-125 ppbFe^{3+}, 2.5-20ppb Mn^{2+} and 2.82-10.87ppm SO_4^{2-}). However, metal mine impacted streams present higher zinc (6.25-1450ppb) and

lead (25.0-157.0ppb) concentrations than coal mine impacted streams (<5ppm Zn^{2+} and lead was below detection limit).

3.4.2.2 Water-sediment interphase

Non-ferrous trace metals recorded in streams impacted by metal mine drainage are undersaturated, hence they do not have the capacity to precipitate as oxides. Nevertheless, Zn^{2+}, Pb^{2+} and Cu^{2+} are found in the sediments, suggesting that adsorption might be the main vector of transport from the water column into the sediments.

From SI calculated in the streams, it is apparent that Fe^{3+} precipitates from the water column as an oxide. This is known to scavenge trace metals, transporting them into the stream bed sediment (Jain and Ram, 1997). However, SS and mineral matter deposited on the stream bed do not vary significantly downstream from the mines, suggesting that ochre precipitation is reduced, limiting the sorption area. When this occurs, Zn^{2+} is found in solution due to competition with other cations for adsorption sites on iron oxides (Smith, 1999). Pb^{2+} and Cu^{2+} have higher affinities for Fe^{3+} oxides and will be adsorbed first (Jean and Bancroft, 1986, Sidle et al., 1991, Jain and Ram, 1997, Smith, 1999), being removed from the water column and transported into the sediment. The presence of Zn^{2+} in the sediment indicates that this is also being transported through sorption mechanisms, although these may be limited.

Zinc and lead are commonly found in sediments collected from streams impacted by metal mine drainage (Table 3.4). Their concentrations recorded at the study sites (640-6440mg/kg Zn^{2+} and 50-62650mg/kg Pb^{2+}) are comparable to those recorded in the

literature (430-9058mg/kg Zn^{2+} and 80-870mg/kg Pb^{2+}) (Table 3.4), and both greatly exceed the sediment quality guidelines suggested by the EA of 12.3mg/kg Zn^{2+} and 35mg/kg Pb^{2+} TEL (Threshold Effect Level) and 315mg/kg Zn^{2+} and 91.3mg/kg Pb^{2+} PEL (Predicted Effect Level) (Chapter 1). Copper can also be found in sediments from streams impacted by metal mine drainage, and Cu^{2+} concentration at the study streams (80-1460mg/kg) are sometimes higher than those reported in the literature (43.24-296mg/kg, (Table 3.4)), and always higher than the TEL (36.7mg/kg) and often PEL (197mg/kg) suggested by the EA. Cadmium was below detection limit at the study sites, and therefore did not exceed the TEL (0.596mg/kg) or PEL (3.53mg/kg).

Manganese is found in higher concentrations in sediments sampled in streams impacted by metal mine drainage (2200-45500mg/kg) than in streams impacted by coal mine drainage (1890-7030mg/kg). In contrast, manganese concentrations in the water column are higher in streams impacted by coal mine drainage (12.5-695ppb) than in streams impacted by metal mine drainage (2.5-20ppb). This suggests that manganese precipitation out of the water column into the sediment is favoured in streams impacted by metal mine drainage. Lee et al. (2002) did not observe manganese precipitation at pH<8, however in both stream types, pH annual average is lower than 8, and only in Cwm Symlog (metal mine) pH readings exceeding 8 were recorded in one occasion (8.04 upstream and 8.5 downstream in winter 2007). Therefore, pH does not seem to be the reason for the difference in manganese precipitation between coal and metal mine drainage impacted streams. Dissolved iron has been observed to interfere with manganese oxide precipitation (Gouzinis et al., 1991, Villinski et al., 2001, Batty et al., 2008), and in metal

mine impacted streams, dissolved iron concentrations are much lower (30-125ppb) than in coal mine drainage impacted streams (87.5-765ppb). This suggests that iron interferences may explain the fact that even though coal mine impacted streams have more manganese in solution than metal mine impacted streams, they have lower manganese concentrations in sediments.

3.5 Conclusion

The present chapter has demonstrated the pollution effects caused by coal and metal mine drainage on water and sediment chemistry of receiving streams, providing the background information necessary to proceed with an ecological study of the affected ecosystems.

Although water chemistry of streams receiving coal mine drainage studied appear not to be severely impacted when compared with other streams studied in the literature, this is not the case with sediments which seem to be highly contaminated. Coal mine pollution appears to be mainly dominated by pyrite (FeS_2) weathering products (Equation 3.1-3.4); since dissolved Fe^{3+}, Fe^{3+} in sediment, SS and acidity increase downstream from the point of mine discharge. However, dissolved Fe^{3+} and pH do not exceed the EQS. Dissolved Mn^{2+} concentration increases significantly downstream from the mine discharge, and exceeds the EQS. Stream waters are oversaturated regarding ferrous compounds, thus iron oxide precipitation from the water column into the river bed is an important process which may have ecological effects. In contrast, Mn^{2+} does not seem to follow the same pattern, and stays in solution keeping concentrations in sediments low. This is probably due to interference with iron present in the stream water, which has been seen to prevent manganese oxide formation (Villinski et al., 2001).

Geochemical data from streams receiving metal mine drainage reflect the presence of sphalerite (ZnS), galena (PbS), and chalcopyrite ($CuFeS_2$) in the catchments, since Zn^{2+}, Pb^{2+} and Cu^{2+} concentrations in sediment increase downstream from the mine discharge. The three metals exceed the sediment quality guidelines suggested by the EA and are

comparable or greater than concentrations recorded in the literature. In the water column, Zn^{2+} (exceeding EQS) is the only metal that presents significant changes in concentration downstream from the mines. This is probably due to higher sorption capacities by Pb^{2+} and Cu^{2+}, which are quickly transported into the sediments (Smith, 1999). Even though lead concentration in water does not significantly increase downstream from metal mine discharge, it exceeds the EQS.

The comparative study of streams polluted by coal and metal mine pollution has identified the differences between the hydrochemical impacts on receiving streams of both mine types, suggesting that ecology may also respond in a different manner. Additionally, the importance of potential sediment toxicity has been highlighted, as heavy metal concentrations in river bed sediments increase significantly downstream from the mine discharge, and lead, zinc and copper exceed the quality guidelines suggested by the EA. This is an important finding as stream sediments are rarely considered in ecological studies. The rest of this project will focus on the relationship between sediment, as well as water chemistry, and communities inhabiting the study streams.

4 ASSEMBLAGE STRUCTURE IN STREAMS IMPACTED BY MINE DRAINAGE

4.1 Introduction

The hydrochemistry of eight Welsh streams impacted by coal and metal mine drainage has been discussed in Chapter 3. Drainage from both types of mine has been suggested to cause deterioration in the quality of stream water and sediment chemistry, although both stream types present physico-chemical dissimilarities. Mine pollution is known to impact the structure of macroinvertebrate aquatic assemblages (Koryak et al., 1972, Tomkiewicz and Dunson, 1977, Jarvis and Younger, 1997, Gray, 1998, Hickey and Clements, 1998, Kelly, 1999, Malmqvist and Hoffsten, 1999, Richardson and Kiffney, 2000, Peeters et al., 2001, DeNicola and Stapleton, 2002, Van Damme et al., 2008). However, the relative importance of water pollution (mainly pH and dissolved heavy metals), sediment toxicity (heavy metals associated with fine particulate), and organism physiology (some species are more sensitive than others, and different functional feeding groups respond to different aspects of the pollution) is not well understood. This study aims to determine the impact of mine drainage pollution (water and sediment) on the structure of macroinvertebrate assemblage in freshwater ecosystems.

4.1.1 The impact of water pollution upon freshwater communities

4.1.1.1 pH effect on organisms

The effect of acidity on freshwater communities has been widely studied, not only in relation to coal mine pollution (e.g. Parsons, 1977, Armitage, 1980, Sasaki et al., 2005), but also acid rain and catchment acidification (e.g. Bell, 1971, Hall et al., 1980, Schindler et al., 1985, Maurice et al., 1987, Ormerod et al., 1987a, Courtney and Clements, 1998, Ledger and Hildrew, 1998, Ledger and Hildrew, 2001, Lepori et al., 2003, Ledger and Hildrew, 2005).

pH can affect aquatic macroinvertebrates via different pathways:

(a) Modifying their physiology, for example disrupting cell membrane transport or changing membrane stability (Hall et al., 1980, Gerhardt, 1993, Camargo et al., 2005);

(b) Increasing macroinvertebrate drift, observed after acidic spells (Ormerod et al., 1987b, Courtney and Clements, 1998);

(c) Decreasing invertebrate emergence, observed in acidic environments (Bell, 1971);

(d) Affecting the availability of suitable food: Algal growth (Ledger and Hildrew, 2001) and detritus conditioning by microorganisms (Nelson, 2000) may be reduced at low pH, subsequently impacting functional groups such as grazers (Hall et al., 1980, Gerhardt, 1993) and shredders (Chapter 5);

(e) Trace metal bioavailability is often increased at low pH, due to three mechanisms that favour the aqueous phase at low pH: (1) changes in the hydrolysis and complexation equilibria, (2) modification of competition between metal ions and H^+ for binding sites, and (3) dependence of sorption capacity on pH (Gerhardt, 1993). However, H^+ can also out-compete heavy metal cations at binding sites in cell membrane carriers and therefore compensate for higher metal availability (Ormerod et al., 1987a, Courtney and Clements, 1998).

An increase in acidity is often associated with coal mine pollution due to H^+ produced in pyrite weathering (Equation 3.1, Chapter 3) (Langmuir, 1997, Smith, 1999, Younger et al., 2002). However in the receiving stream environment it is difficult to discern the effect of pH from that of dissolved heavy metals and ochre deposition, as they normally occur simultaneously (Chapter 3). When pH effect has been isolated in mine polluted streams, it has been associated with decreased benthic species richness but with no change in biomass (expressed as mg dry weight/m^2) (Tomkiewicz and Dunson, 1977) or total abundance (Koryak et al., 1972), whereas extensive ochre deposition in combination with low pH appears to have a negative effect on both species richness and total abundance (Koryak et al., 1972, Jarvis and Younger, 1997).

Tolerance to low pH appears to vary between insect taxa. Plecoptera have been defined as an opportunistic order resistant to moderately acidic environments (Tomkiewicz and Dunson, 1977). Species of *Nemoura* (Plecoptera) are known to be common in European acidic streams (Koryak et al., 1972), probably because they are not obligate detritivores and take advantage of competitive release, occupying grazer niches when potential competitors are absent (Ledger and Hildrew, 2000, Ledger and Hildrew, 2001, Ledger and Hildrew, 2005). Chironomidae are also thought to be acid tolerant (Van Damme et al., 2008). Specialised grazers, such as many Ephemeroptera, have been shown to be very sensitive to decreases in pH (Ledger and Hildrew, 2005) and are often absent when pH is lower than 5 (Rosemond et al., 1992).

4.1.1.2 Trace metal effect on organisms

Organismal uptake of trace metals occurs via three main pathways; water, sediment and food. As many invertebrates ingest sediment particles containing microalgae, fungi and bacteria while feeding, distinction between biotic (food) and abiotic (sediment) input is difficult (Gerhardt, 1993).

All aquatic invertebrates take up trace metals from the environment to some extent (Rainbow, 2002). However, bioaccumulation (higher metal concentrations in organisms than in the surrounding environment) only occurs if the uptake rate is higher than the excretion and detoxification rate of the organism (Rainbow, 2002). At the food web level, this may lead to organisms in higher trophic strata accumulating metals via the food web (biomagnification) (Gerhardt, 1993).

Toxicity from trace metals is mainly associated with biochemical reactions involving:

(a) Competitive blockage of a functional group or macromolecule at the cell membrane, which can disrupt transport and membrane stability (Gerhardt, 1993);

(b) Displacement of other cations. Zinc can cause cross linking of DNA-molecules, inhibiting transcription processes, and copper can depress the electrical response of the nervous cells (Gerhardt, 1993);

(c) Conformational change in proteins, for example copper can bind to certain enzymes and inhibit their action (Flemming and Trevors, 1989).

Physiological effects from heavy metal toxicity are manifested mainly as hypoxia (deficiency of oxygen reaching the body tissues), caused by a reduction in gas exchange due to coagulation and precipitation of mucus or cytological damage (Koryak et al., 1972, Sridhar et al., 2001, Niyogi et al., 2002a).

Uptake and bioaccumulation of trace metals does not always result in a toxic effect, as they can be stored as non-toxic species or bound to metallothionein (Gerhardt, 1993). Metal toxicity can depend on biotic factors: size and life stage (Kiffney and Clements, 1996), feeding characteristics such as gut volume, gut passage time and gut pH (Kelly, 1999), and alimentation habits (i.e. functional feeding groups) (Kelly, 1999). Hence, tolerance to trace metal pollution may vary between species and functional groups. Ephemeroptera have been classified as one of the taxa most sensitive to trace metals, whereas Chironomidae appear to form one of the most tolerant groups (Arnekleiv and

Storset, 1995, Hickey and Clements, 1998, Richardson and Kiffney, 2000, Hickey and Golding, 2002, Van Damme et al., 2008).

Tolerance to pollution can also be developed through adaptation (genetically based) when the population has lived under stress for several generations (Gerhardt, 1993, Morgan et al., 2007), or acclimation, when a population is pre-exposed to pollution (Gerhardt, 1993, Admiraal et al., 1999, Peeters et al., 2001).

The toxic effects of metal mine drainage are often attributed to heavy metals such as copper and zinc in water (Gray, 1998, Admiraal et al., 1999, Malmqvist and Hoffsten, 1999, Nelson, 2000, Richardson and Kiffney, 2000, Niyogi et al., 2001, Peeters et al., 2001, Sridhar et al., 2001, Hickey and Golding, 2002, Niyogi et al., 2002a, Carlisle and Clements, 2005). However, heavy metals can also be transferred into the sediments (Chapter 3) and potentially impact benthic organisms. This sediment-benthos association is not yet fully understood.

4.1.2 The impact of polluted sediments upon freshwater communities

Sediments form a sink for heavy metals due to the adsorption capacity of clay, organic matter and other solids such as ochre flocs (Smith, 1999, Peeters et al., 2001) (Chapter 3). Trace metal loaded sediments may pose a threat for benthic organisms, but they can also be a source of pollutants when physico-chemical properties of the stream (e.g. low

pH, high discharge) favour desorption of heavy metals or erosion of contaminated sediment (Bervoets et al., 1997, Kelly, 1999, DeNicola and Stapleton, 2002).

Benthic organisms are able to take up metals while burrowing in sediments or when feeding from particulate matter (Bervoets et al., 1997, Bervoets et al., 1998). Nevertheless, a direct link between sediment trace metal concentration and an adverse effect on invertebrates has not always been found (Bervoets et al., 1998, Van Damme et al., 2008). Some authors suggest that invertebrates collect trace metals from sediment pore water or from the boundary layer between the surface of the sediment and the overlying water column (Bervoets et al., 1998, Courtney and Clements, 2002). It is probably a combination of substratum, water and organism physiology that play an important role in the biogeochemical cycling of metals from the sediment (Kelly, 1999). Thus, it becomes apparent that more study is needed to fully understand the role of all these factors on the toxicity of mine pollution, and trace metals associated with it, on freshwater ecosystems.

Sediments may also integrate long term metal exposure of lotic ecosystems, whereas metal concentrations in water are generally more variable (Van Damme et al., 2008). Gray (1998) observed a seasonal effect on mine discharge (l/s) and metal discharge rates (kg/s), both reaching their maximum in wetter months (February) and minimum in drier periods (October). Pollution input from spoil heap runoff has also been seen to vary seasonally (Younger et al., 2002, Gandy and Younger, 2007, Canovas et al., 2008). Spoil heaps that do not develop a water table appear to produce maximum contamination during wet periods, when surface runoff dissolves metals from spoil material and

transports them to the receiving water body. On the contrary, some spoil heaps are big enough to develop a localised water table, and in this case, high rainfall dilutes the drainage from the spoil heaps, resulting in lower trace metal concentrations and acidity during wet periods (Gandy and Younger, 2007). Additionally, these processes may be counteracted by greater dilution in reaching the receiving stream during high flow events, which often coincide with wetter periods (Canovas et al., 2008). Therefore, trace metal concentrations in water may be highly variable depending on the hydrology of the streams, and sediment toxicity may be a better predictor of benthic species variation. However, sediment analysis is often disregarded in routine monitoring work.

The detrimental impact of polluted sediment in mine drainage is not always associated with trace metal toxicity. In streams impacted by coal mine drainage, extensive deposition of ochre often covers the stream bed (Chapter 3). This may cause a series of physical effects on the benthic community by (a) limiting food resources by lowering primary productivity (Koryak et al., 1972, Hall et al., 1980) or coating detritus preventing grazer access (Nelson, 2000) (Chapter 5), (b) clogging of invertebrate gills (Koryak et al., 1972, Hall et al., 1980) (Plate 4.1), and (c) reducing habitat availability due to fine sediment deposition (Rabeni et al., 2005). However, ochre flocs have high adsorption capacity for heavy metals dissolved in the water (Parkman et al., 1996, Jain and Ram, 1997, Smith, 1999) (Chapter 3), thus their toxic effect may also be relevant.

4.2 Aims and objectives

Two hypotheses have been established based on the literature and the chemical study of the sites: (1) communities inhabiting streams impacted by coal mine drainage respond to pH and ochre deposition; (2) organisms inhabiting metal mine impacted streams are influenced mainly by trace metal concentration in water and sediment.

The main objectives of the present study are:

a) Determine if abandoned mine drainage impacts on the structure of the aquatic community in receiving streams;

b) Detect differences in benthos response to coal and metal mine drainage pollution

c) Assess the relative importance of water related variables and sediment related variables in predicting macroinvertebrate community structure at the sampled streams;

d) Relate environmental variables to changes in benthos composition.

4.3 Methodology

4.3.1 Species data

Ephemeroptera, Plecoptera, Trichoptera and Chironomidae (EPTC) were sampled upstream and downstream from each of the eight studied mines (4 coal mines and 4 metal mines, Figures 2.1-2.9, Chapter 2) and identified to species level (Chapter 2). A complete list of the species identified in the study streams can be found in Appendix B.

In order to compare communities upstream and downstream from mine discharges, diversity indices have been calculated, as they have been described as measurements of ecological community wellbeing (Magurran, 1988). A number of diversity indices exist but little consensus has been reached on the best one for ecological studies. Species richness (count of the number of species present in the sample), is widely used in ecology, because it is simple to measure, easy to interpret and it is statistically and ecologically sound; however, it is highly influenced by sample size and misses dominance shifts in communities (Magurran, 1988). Shannon-Wiener index, on the other hand, accounts for evenness and species richness, is less sensitive to sample size, but it is highly criticised due to its poor ability to discriminate between samples (Magurran, 1988). Evenness includes species abundance in the calculations, but it is highly affected by sampling variations (Magurran, 1988). In summary, no single index can be selected for its best performance, therefore, in the present study several indices are calculated: species

richness (S), Shannon-Wienner index (H) and evenness (E). “Species, Diversity and Richness” Pisces program was used to calculate species richness and Shannon-Wienner index, whereas evenness was calculated following Pielou’s formula (Heino, 2008):

$$E=H/\log(S)$$

4.3.2 Environmental data

The present study relates the chemistry of eight Welsh streams impacted by mine drainage to the macroinvertebrate community inhabiting them. The hydrochemical analysis of the study streams has been discussed in Chapter 3, but for the present study only those variables that significantly change downstream from the mine discharge and that are not correlated have been used (Table 4.3 and 4.4). Streams impacted by coal mine drainage are characterised by an increase in dissolved iron, manganese, sulphate, suspended solids (ochre flocs), and iron concentration in sediments; and a decrease in pH (Table 4.1). Streams impacted by metal mine drainage are characterised by an increase in zinc, sulphate and nitrate concentrations in water and elevated concentrations of zinc, lead and copper in sediments (Table 4.2).

Environmental data from metal mine drainage impacted streams had several outliers and did not present a normal distribution. In order to fulfil the assumptions of homogeneity and normality required for valid statistical analysis (Leps and Smilauer, 2007), the data set was log transformed before proceeding with ordination analysis.

Table 4.1 Annual mean (± SD, n=4) of variables that significantly change downstream from coal mine drainage discharge in four streams from South Wales between summer 2006 and spring 2007

	Aberbeiden		NantyFyllon		Clyne		Cwm Gross	
	U/S	D/S	U/S	D/S	U/S	D/S	U/S	D/S
Sulphate (ppm)	24.9±16.6	118.1±106.7	15.78±7.6	42.3±20.7	72.67±31.4	68.7±36.1	18.5±20.6	64.4±29.6
Sodium (ppm)	8.3±1.0	17.4±5.6	5.7±0.5	5.9±0.5	11.3±1.0	10.3±1.23	4.9±0.7	50.7±33.4
Magnesium (ppm)	3.2±0.7	14.5±7.9	3.9±1.4	7.1±2.6	13.0±4.0	12.3±4.0	1.8±0.3	7.4±3.8
Potassium (ppm)	1.2±0.01	3.4±1.3	2.2±0.5	2.9±1.5	4.8±0.6	3.8±0.6	0.6±0.1	6.3±3.9
Calcium (ppm)	7.7±2.9	32.3±25.5	5.1±2.1	13.4±9.1	40.4±18.1	38.6±19.1	5.8±2.0	14.1±9.2
Iron (ppb)	107.5±75.9	102.5±56.2	765.0±336.9	107.5±171.5	353.3±242.12	190.0±209.4	207.5±249.5	87.5±71.8
Magnesium (ppb)	25.0±33.2	695.0±731.3	35.0±57.5	76.7±86.2	60.0±120.0	157.4±177.6	12.5±15.0	107.5±75.9
pH	7.23±0.2	7.1±0.1	7.2±0.56	6.9±0.4	7.4±0.2	7.1±0.1	6.7±0.45	6.8±0.2
TDS (ppm)	91.2±16.6	280.5±132.0	62.12±7.3	120.5±42.9	257.2±72.9	258.4±69.2	56.7±11.8	246.6±137.3
EC (µS)	134.6±24.23	401.4±185.0	90.7±12.12	176.7±62.23	368.8±103.4	370.34±97.9	83.6±18.3	353.3±194.78
SS (ppm)	3.2±2.6	7.4±3.3	0.5±1.0	2.4±1.3	4.7±3.6	6.7±4.4	3.6±1.1	7.67±2.9
Iron in sediments (mg/kg)	51000±21900	159780±172000	29500±4000	37900±49000	54500±18500	57300±74100	56700±10300	45700±39600

Table 4.2 Annual mean (± SD, n=4) of variables that significantly change downstream from metal mine drainage discharge in four streams from Mid Wales between summer 2006 and spring 2007

	Dylife		Frongoch		Cwm Ystwyth		Cwm Symlog	
	U/S	D/S	U/S	D/S	U/S	D/S	U/S	D/S
Chloride (ppm)	8.1±2.2	9.6±2.3	9.5±0.8	9.9±1.5	6.0±0.7	7.3±2.2	12.0±2.1	12.4±2.5
Nitrate (ppm)	3.1±2.4	3.7±1.7	2.3±1.5	2.6±1.1	0.8±0.5	1.2±0.5	1.2±0.5	3.8±1.4
Sulphate (ppm)	2.8±0.6	8.7±5.1	6.3±1.4	10.1±3.0	3.7±0.8	5.8±2.6	5.5±0.6	10.9±1.2
Zinc (ppb)	87.5±175.0	942.5±944.7	35.5±39.9	1450.0±1190.2	6.3±9.5	410.0±318.4	22.5±45.0	64.3±123.9
EC	55.9±5.6	74.6±16.3	86.6±21.1	91. 6±20.0	46.1±11.4	47.3±11.4	66.4±6.7	109.8±19.2
TDS (ppm)	38.3±3.7	51.1±10.9	59.2±13.7	69.2±14.2	31.7±8.0	32.5±7.6	45.3±5.4	59.9±29.8
Zinc in sediments (mg/kg)	5500±8000	6400±2500	2100±1400	4200±1900	600±600	2600±1900	900±700	1900±1200
Lead in sediments (mg/kg)	31700±51700	62700±1700	2400±2100	25700±19300	100±100	7900±5700	18600±20900	22800±10100
Copper in sediments (mg/kg)	800±1100	1500±400	BD	BD	BD	BD	BD	900±1300

Table 4.3 Environmental variables representing coal mine drainage pollution

Variable used	Correlated variables
Iron in water	Manganese in water
SS	Sulphate, TDS, calcium, potassium, magnesium, sodium and EC
pH	None
Iron in sediments	None

Table 4.4 Environmental variables representing metal mine drainage pollution

Variable used	Correlated variables
Zinc in water	None
EC	Sulphate, TDS and chloride
Nitrate	None
Zinc in sediments	Copper and lead in sediments

4.3.3 Statistical analysis

4.3.3.1 Ecosystem structure paired analysis

Paired differences (upstream and downstream) of species richness, Shannon-Wiener index, abundance and evenness were calculated using the Wilcoxon sign rank test. A paired test was chosen to account for differences between sites.

4.3.3.2 Multivariate statistics

Seasonal differences have been observed on the response of benthos to coal mine drainage pollution, therefore the data were analysed separately for autumn 2006 and winter 2007, and summer 2006 and spring 2007. This phenomenon was not observed in metal mine communities, hence these data were analysed as one single set.

The relationship between environmental variables and ecological data was initially tested using GAM (Poisson distribution with log link and 4df) on total abundance and species richness. Subsequently, CANOCO for windows 4.5 was used for ordination analysis of species distribution. DCCA was carried out to decide whether to use unimodal or linear ordination methods in the analysis. The gradient length obtained, measures the beta diversity in community composition along the ordination axes (Leps and Smilauer, 2007), testing the likelihood of there being a linear or unimodal response of species distribution to environmental variables. When the longest gradient was shorter than 3, Redundancy Analysis (RDA) was adopted. If it was longer than 4, Canonical Correspondence Analysis

(CCA) was used (Leps and Smilauer, 2007). When the longest gradient length was between 3 and 4 the most significant method was chosen (Chapter 2).

Forward selection of explanatory variables was used to find a subset of variables that represents the relationship between species and the environment (Leps and Smilauer, 2007). Monte Carlo permutation test assessed the usefulness of each explanatory variable added to the ordination model, variables with $p<0.05$ were taken as significant.

Variance partitioning was used to discern between the effects of water and sediment chemistry. The percentage of species variation explained by water chemistry was estimated from the sum of eigenvalues using water chemistry as environmental variables and sediment variables as covariables in the analysis (Legendre and Legendre, 1998). Similarly, the effect of sediment chemistry on the aquatic community was estimated from the sum of eigenvalues using sediment chemistry as environmental variables and water chemistry as covariables.

Contour graphs representing species richness variation with environmental variables were drawn using a GAM in CanoDraw for windows. Analyses with $p<0.05$ were used for the study.

4.4 Results

4.4.1 Coal mine impacted streams

4.4.1.1 Changes in assemblage structure downstream from coal mine drainage discharge

Table 4.5 depicts the results of Wilcoxon signed rank test, demonstrating that Ephemeroptera, Plecoptera, Trichoptera and Chironomidae (EPTC) species number ($z=-2.819$, $p=0.005$) and abundance ($z=-3.351$, $p=0.001$) significantly decrease downstream from the coal mine discharge at the study sites, whereas evenness ($z=-1.667$, $p>0.05$) and Shannon-Wiener index ($z=0$, $p>0.005$) do not change significantly. Ephemeroptera, Plecoptera and Trichoptera (EPT) species number ($z=-2.986$, $p=0.003$), abundance ($z=-3.294$, $p=0.001$), evenness ($z=-3.294$, $p=0.001$) and Shannon-Wiener index ($z=-2.556$, $p=0.011$) are significantly reduced downstream from coal mine drainage discharge. On the other hand, Chironomidae community structure does not seem to be affected by coal mine drainage.

Table 4.5 Wilcoxon signed rank test paired comparison (upstream, downstream) of ecosystem structure (EPTC, EPT and Chironomidae) in coal mine impacted streams from summer 2006 until spring 2007 (n=32)

Taxa		Species number	Abundance	Evenness	Shannon-Wiener index
EPTC	Z	-2.819	-3.351	-1.667	0
	p	0.005*	0.001*	>0.05	1
EPT	Z	-2.986	-3.294	-3.294	-2.556
	p	0.003*	0.001*	0.001*	0.011*
Chironomidae	Z	-1.137	-1.600	-0.178	-1.245
	p	>0.05	>0.05	>0.05	>0.05

* downstream values significantly lower than upstream

4.4.1.2 Relationship between ecology and coal mine drainage physico-chemical characteristics

The relationship between environmental variables and ecological data was initially tested using a GAM (Poisson distribution with log link and 4 df) on total abundance and species richness. This test indicates that all variables considered in the analysis (Fe, EC, SS and Fe in sediments) significantly explain the variation in total abundance ($p<0.001$) and species richness ($p<0.001$) in streams impacted by coal mine drainage. As seasonal variation was observed in the response of macroinvertebrates to coal mine pollution, the rest of the analysis is done with autumn and winter data separated from summer and spring.

4.4.1.2.1 Autumn 2006 and winter 2007

Figure 4.1 depicts the variation in abundance in relationship to environmental variables, demonstrating a significant negative effect of coal mine pollution on EPTC (F=4.87, p=0.023) in the colder months. Suspended sediment (SS) appears to be the most detrimental variable at the coal mine polluted streams, according to the length and direction (perpendicular to isolines) of the arrows in the diagram. A positive effect of pH depicted in the graph is a result of the inverse meaning of this variable in relation to acidity, hence Figure 4.1 suggests that low pH causes a negative effect. In summary, all four variables chosen as representative of coal mine pollution (section 4.2.1) appear to impact upon EPTC abundance at the study sites in autumn 2006 and winter 2007.

Environmental variables considered for the analysis can explain 88% of the species variation at the study sites. Additionally, variation partitioning indicates that sediment related variables (SS and iron in sediment) are slightly more relevant (42%) than water related variables (dissolved iron and pH) (39%) when explaining EPTC species variation in the study streams (Figure 4.2). Furthermore, forward selection demonstrated that SS is the most important variable explaining species distribution in streams impacted by coal mine drainage (F=1.92, p=0.004) (Table 4.6).

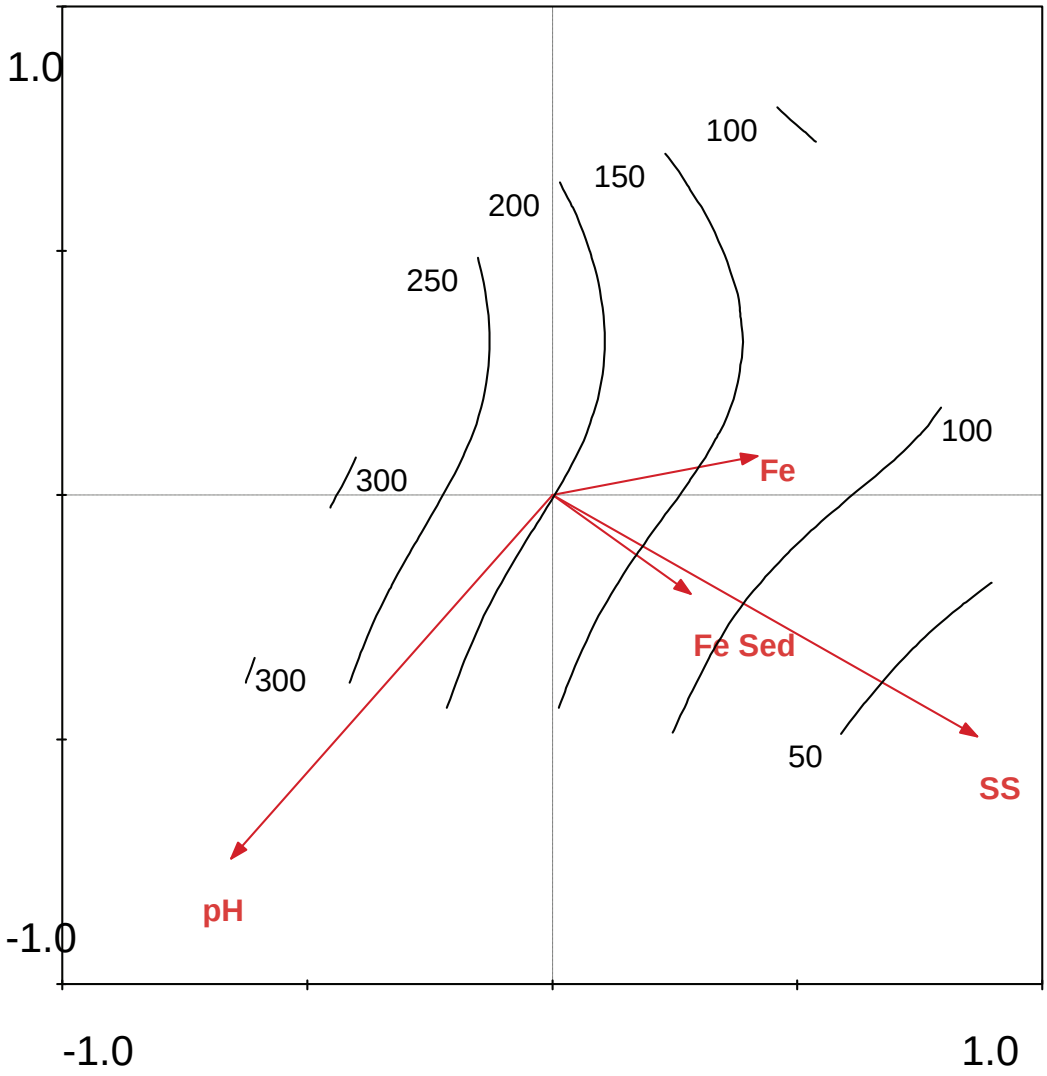


Figure 4.1 Isolines of EPTC abundance in coal mine impacted streams (autumn 2006 and winter 2007) plotted in the CCA ordination diagram with the effect of environmental variables (SS: Suspended solids; Fe sed: iron concentration in sediment; Fe: iron concentration in water)

Table 4.6 Forward selection of environmental variables (n=16) explaining EPTC species variation in coal mine polluted streams in autumn 2006 and winter 2007

Variable	Lambda 1	F	p
SS	0.36	1.92	0.004
pH	0.27	1.47	>0.05
Fe	0.15	0.79	>0.05
Fe sediments	0.10	0.52	>0.05

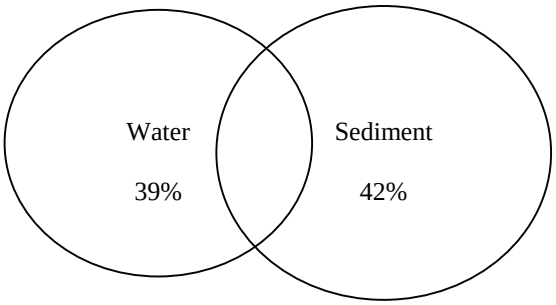


Figure 4.2 Partitioning of variance of species composition (n=16) explained by water and sediment related variables in coal mine impacted streams during autumn 2006 and winter 2007

4.4.1.2.2 Summer 2006 and Spring 2007

Figure 4.3 depicts a significant (F=6.73, p=0.03) impact of coal mine drainage on EPTC abundance in summer and spring. Sediment iron concentration and pH appear to be the dominant variables in explaining abundance variation in streams. SS, acidity, iron concentrations in water and sediment appear to impact on EPTC abundance.

Environmental variables selected for the analysis can explain 55% of the EPTC species variation during summer 2006 and spring 2007. Variation partitioning analysis indicates that water (30%) is more important than sediment (19%) when explaining EPTC species distribution at the study streams (Figure 4.4). However, forward selection did not choose any variable as significantly more important when explaining this pattern (Table 4.7).

Table 4.7 Forward selection of environmental variables (n=16) explaining species variation in coal mine impacted streams (summer 2006 and spring2007)

Variable	Lambda 1	F	p
pH	0.27	3.03	>0.05
Fe Sediment	0.21	2.75	>0.05
SS	0.05	0.58	>0.05
Fe	0.02	0.728	>0.05

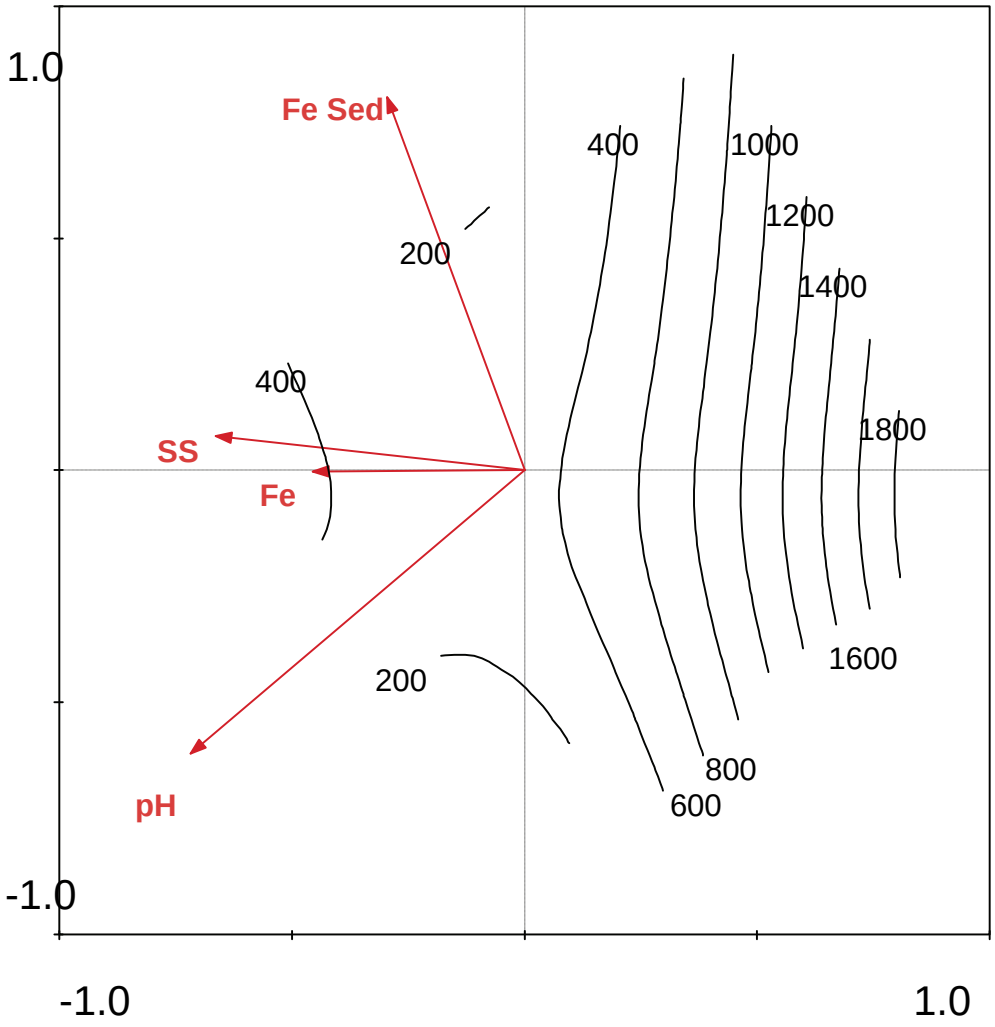


Figure 4.3 Isolines of EPTC abundance in coal mine impacted streams (summer 2006 and spring 2007) plotted in the RDA ordination diagram with the effect of environmental variables (SS: Suspended solids; Fe sed: iron concentration in sediment; Fe: iron concentration in water)

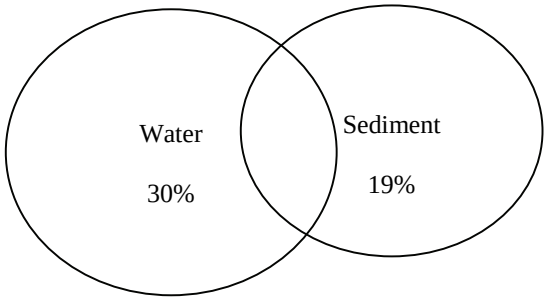


Figure 4.4 Partitioning of variance of species composition (n=16) explained by water and sediment related variables in coal mine impacted streams during summer 2006 and spring 2007

4.4.2 Metal mine impacted streams

4.4.2.1 Changes in community structure downstream from metal mine drainage discharge

Table 4.8 depicts paired comparisons of several community indices. EPTC Shannon-Wiener index (z=-1.241, p=0.016) and Evenness (z=-3.051, p=0.002) significantly decrease downstream from metal mine drainage discharge, whereas species number (z=-1.480, p>0.05) and abundance (z=-1.241, p>0.05) do not present significant changes. Chironomidae present a significant reduction in abundance (z=-2.669, p=0.008), species number (z=-2.806, p=0.005) and Shannon-Wiener index (z=-2.329, p=0.020). Whereas only Shannon-Wiener index (z=-2.884, p=0.004) is reduced in EPT.

Table 4.8 Wilcoxon signed rank test paired comparison (upstream and downstream) of metal mine impacted streams ecosystem structure indices (EPTC, EPT and Chironomidae) between summer 2006 and spring 2007 (n=32)

		Species number	Abundance	Evenness	Shannon-Wiener index
EPTC	Z	-1.480	-1.241	-3.051	-2.414
	p	>0.05	>0.05	0.002*	0.016*
EPT	Z	-0.370	-1.810	-1.862	-2.844
	p	>0.05	>0.05	>0.005	0.004*
C	Z	-2.806	-2.669	-1.761	-2.329
	p	0.005*	0.020*	0.008*	>0.05

* downstream values significantly (p<0.05) lower than upstream

4.4.2.2 Relationship between assemblage structure and metal mine drainage chemical characteristics

GAM (Poisson distribution with log link and 4df) demonstrates that all the variables considered for the analysis (Zn, nitrate, EC and Zn in sediments) significantly explain the variation in EPTC total abundance ($p<0.001$) and species richness ($p<0.001$) in streams impacted by metal mine drainage.

Figure 4.6 depicts two components impacting on the Shannon-Wiener index of streams receiving metal mine drainage (a) zinc in water and sediment, with dissolved zinc exerting a stronger effect, (b) nitrate and conductivity, where nitrate is the dominant variable.

Environmental variables selected to study the relationship between EPTC structure and the environment, explain only 21% of the total species variation. Variation partitioning indicates that water chemistry (conductivity, nitrate and zinc concentrations) (18%) is the main factor explaining species distribution, whereas sediment chemistry (zinc, lead and copper concentration) takes a lesser role (2%) (Figure 4.5). Additionally, nitrate was selected as the most influential variable ($F=3.46$, $p=0.032$) (Table 4.9).

Table 4.9 Forward selection of environmental variables (n=32) explaining species variation in metal mine impacted streams

Variable	Lambda 1	F	p
Nitrate	0.12	3.46	0.032
Zinc	0.03	0.86	>0.05
Conductivity	0.02	0.60	>0.05
Zinc in sediment	0.02	0.59	>0.05

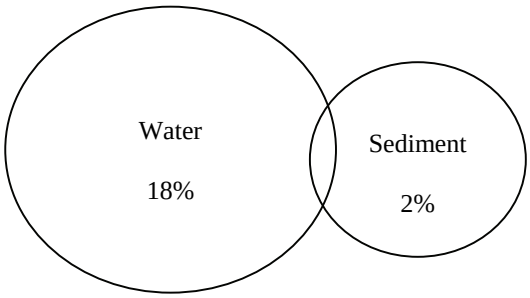


Figure 4.5 Partitioning of variance of species composition (n=32) explained by water and sediment related variables in metal mine impacted streams

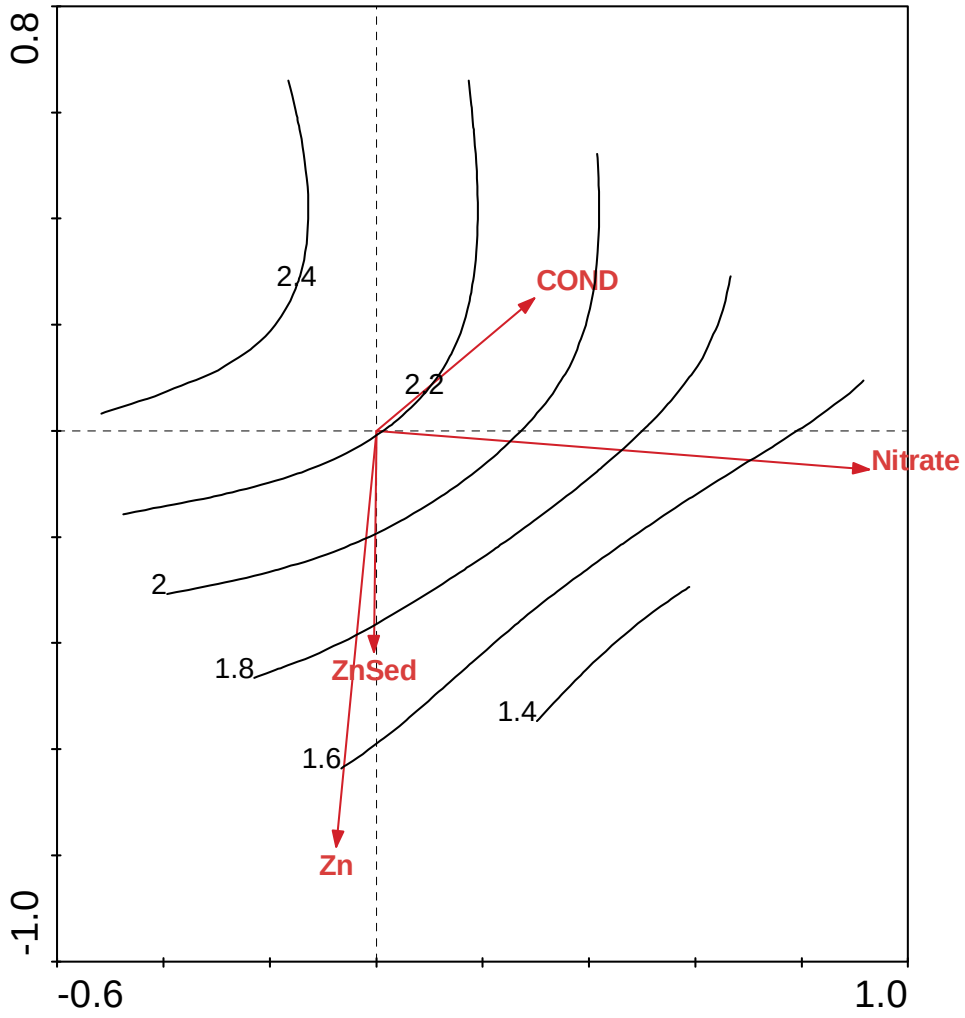


Figure 4.6 Isolines of EPTC Shannon-Wiener index in metal mine impacted streams plotted in the RDA ordination diagram with the effect of environmental variables (Zn: zinc concentration in stream water, Zn Sed: zinc concentration in sediments, Nitrate: nitrate concentration in stream water, COND: conductivity)

4.5 Discussion

4.5.1 Ecological structure of coal mine drainage impacted streams

Coal mine drainage has been observed to impact on aquatic communities (e.g. Koryak et al., 1972, Feldman and Connor, 1992, Gray, 1998), which is corroborated by the present study, with EPTC abundance and species richness reduction downstream from the mine discharges. EPT appear to be more sensitive than Chironomidae to coal mine pollution, the reasons behind the differences between both groups are discussed in this section.

Iron in sediment and water, pH and suspended sediments (SS) were selected as variables representative of coal mine drainage pollution for this study (Section 4.2.). Collectively, they can explain most (88% in cold months and 55% in warmer months) of the species variation. Sediment related variables (SS and iron in sediments) (explaining 42% of species variation) appear to be more important than water related variables (iron, pH and conductivity) (explaining 39% of species variation) when explaining species distribution at the study streams during autumn 2006 and winter 2007. SS appears to be the most important factor conditioning benthos composition in the sampled streams during the colder seasons. SS are thought to cause a physical effect on benthos related to:

(a) direct effect on organisms by clogging their gills (Koryak et al., 1972, Hall et al., 1980) (Plate 4.1);

(b) reduction of food availability as algae (Koryak et al., 1972, Ormerod et al., 1987b, Sridhar et al., 2001, Niyogi et al., 2002a, Niyogi et al., 2002b) or detritus (Nelson, 2000, Niyogi et al., 2001, Sridhar et al., 2001) (Chapter 5);

(c) loss of habitat (Rabeni et al., 2005).

Additionally, as discussed in Chapter 3, SS are thought to be predominantly in the form of iron oxides, which are known to adsorb trace metals present in the water column (Parkman et al., 1996, Jain and Ram, 1997, Smith, 1999). Hence, ochre flocs suspended in the water column and heavy metals associated with them may cause not only a physical effect but also toxicity to organisms inhabiting the streams.

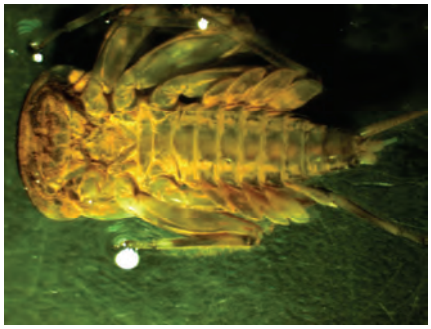


Plate 4.1 Ventral view of *Ecdyonurus insignis* (Ephemeroptera) sampled in a site polluted by coal mine drainage, its gills and body are covered with ochre (Source: Original)

At this point, it must be remembered that SS also represent the electrolyte characteristics of the water (Section 4.2.1), so their negative effect suggested by this analysis may be related to an impact of higher ion concentration in the water.

The present study suggests that the effect of coal mine drainage upon aquatic communities in warmer seasons (summer 2006 and spring 2007) might be limited or masked by:

(a) Better conditions, such as food and temperature during warmer months (Jeyasingham and Ling, 2000);

(b) Faster larval development in warm environments and hence shorter contact time with pollution in the stream (Servia et al., 2004);

(c) Effect of life cycles. Many univoltine invertebrates remain in resting stage (e.g. egg-dipause) during cold months (Hynes, 1970), thus macroinvertebrates are often less abundant in winter than in summer;

(d) Dominance of Chironomidae (some of which are tolerant to fine sediments) in warm seasons, whereas Plecoptera is the dominant order in cold seasons (Appendix C).

This seasonal variation in the effect of coal mine pollution could also be due to changes in water or sediment chemistry as mine drainage flow or composition may vary seasonally. Seasonal variations have not been observed in the hydrochemical data obtained in this study, although variation of this nature maybe missing because sampling was not

designed with this specific purpose (Chapter 3). Seasonal fluctuations in water chemistry from streams impacted by mine drainage have been observed, low discharge periods (low rainfall) typical of summer in the UK, having a higher impact on macroinvertebrates (Herricks and J., 1972, Herricks and Cairns, 1977). This is probably related to less water in the stream available for dilution of mine drainage (Canovas et al., 2008), and greater metal concentrations observed during dry periods in the input from spoil heaps that develop a water table (due to decreased runoff for dilution) (Canovas et al. 2008, Gandy et al., 2007, Younger et al., 2002). In contrast, a decrease in mine discharge during dry months has been observed before (Gray, 1998), and spoil heap (without water table) input is also reduced as runoff deceases during dry periods (Younger et al., 2002, Gandy et al., 2007, Canovas et al., 2008). Thus, seasonal effect on stream hydrochemistry is a complicated issue that requires intensive sampling and analysis and is out of the scope of the present study.

The environmental variables appear to explain less of the species variation in warmer months than in colder months, which might suggest that other factors such as invertebrate life cycles, food availability and contact time with pollutants not included in the analysis may play an important role in these months.

The impact of water related variables on lotic ecosystem (explaining 30% of species variation) seems to be more relevant than the sediment effect (explaining 19% of species variation) in warmer months. This shift may be due to the dominance of Chironomidae during warmer months. Chironomids are ubiquitous in all freshwater habitats (Pinder, 1983), including fine sediments (Spanhoff et al., 2007). In these environments with high

SS, some species may benefit from competition and/or predation release, and thus thrive. Additionally, acidity appears to cause a positive effect on EPTC abundance (Figure 4.5), which might be due to dominance of tolerant species, such as chironomids (Van Damme et al., 2008), under these conditions due to competition or predator release. However, no variable has been selected as significantly more important when explaining benthic species distribution in coal mine impacted streams during summer 2006 and spring 2007, suggesting that the effect is reduced or limited.

In summary, coal mine drainage significantly affects macroinvertebrate assemblage in cold months. This impact could be mainly attributed to sediment related variables (explaining 42% of species variation) rather than water related variables (explaining 39% of species variation). Amongst the sediment related variables, SS seems to be the most influential factor in benthic faunal depletion, occurring mainly through physical effects. However, in warmer periods, the impact of coal mine drainage on freshwater insects appears to be reduced, probably due to more favourable conditions and dominance of Chironomidae, some of which are tolerant of fine sediment (Spanhoff et al., 2007).

4.5.2 Ecological structure of metal mine drainage impacted streams

This study demonstrated a negative effect of metal mine drainage on aquatic communities, by reducing species evenness (Pielou's formula) and species diversity (Shannon-Wiener index). Changes in species diversity caused by metal mine drainage have been observed, often in conjunction with decreases in macroinvertebrate abundance (Koryak et al., 1972, Feldman and Connor, 1992, Gray, 1998, Hirst et al., 2002), but not always (Malmqvist and Hoffsten, 1999). Reduction of organism diversity and evenness, but not abundance, observed in this work indicates a dominance of tolerant species downstream from metal mine outflows. Malmqvist and Hoffsten (1999) also observed decreases in species diversity but not abundance in streams impacted by metal mine drainage; however, Ephemeroptera and Plecoptera appeared to be the most sensitive taxa in their study, and this is not the case in the present study. Structural changes presented by EPT and Chironomidae from metal mine impacted streams suggest that Chironomidae is more sensitive in these environments. Possible reasons for this are investigated in this section.

Environmental variables considered for the present study only explain 21% of the species variation, the remaining variance might be attributed to ecological relationships within the ecosystem (e.g. predation, competition and facilitation), chemical characteristics of the stream not measured (e.g. organic matter in the water or sediment, pore water metal concentrations), or other factors not accounted for (e.g. habitat and vegetation cover).

However, water related variables (conductivity, nitrate and zinc concentration) can explain more of the species variation than sediment chemistry (zinc concentration, which also represents lead and copper because of correlation between variables). The importance of water chemistry in explaining species distribution in metal mine impacted streams has also been observed by Van Damme et al. (2008), who attributed it to both the greater contact of benthic organisms with overlying and interstitial water than with sediments, and to high sediment instability in running waters due to transport and erosion processes. However, similar environments are found in streams impacted by coal mine drainage, and there sediment related variables appear to be better predictors of species variation. Therefore, the main reason may be linked to the presence of suspended sediments (SS) in the water column. Increase in SS is one of the main impacts caused by coal mine drainage on the receiving water course due to extensive iron oxide precipitation (Chapter 3 and Table 4.1), however, non-ferrous metals (dominant in metal mine drainage) do not typically form oxides, and certainly extensive mineral matter deposition was not observed at the study sites (Chapter 3 and Table 4.2). Therefore, it seems that when there is no physical negative effect from SS, water chemistry is the dominant factor in species variation. The aspects of water chemistry in metal mine impacted streams that are detrimental to freshwater communities are discussed below.

No seasonal differences were observed in the response of macroinvertebrates in streams polluted by metal mine drainage, contrasting the results found in streams impacted by coal mine drainage. This may be due to a difference in the nature of the impact. The

physical effect of SS (coal mines) may be counteracted by seasonal changes, whereas, invertebrates response to water toxicity (metal mines) might not be overcome by subtle seasonal changes.

For the present study, nitrate concentration (0.22-6.04ppm) was selected as the most important variable explaining the negative effect of metal mine drainage on aquatic communities, even though it never exceeds the EQS (Environmental Quality Standards) of 17.5ppm (Chapter 1, Table 1.1). Despite nitrate concentration increase downstream from metal mine discharge, this nutrient may not be directly linked to metal mines and it probably originates in the surrounding agricultural land, where grazing is the main land use (Chapter 3).

Algal growth has been seen to increase with nitrate at similar concentrations to the present study (2-3ppm) (Camargo et al., 2005), potentially leading to an enhancement in the whole community due to more food availability. However, in the present study, algal growth downstream from metal mines is reduced (Chapter 5), suggesting that negative effects of metal mine drainage pollution overcome possible positive effects due to nitrate enrichment.

Alternatively, nitrate concentrations similar to those at the study streams (2ppm $NaNO_3$) have been seen to reduce *Chironomus teppori* oviposition (Stevens et al., 2003). Chironomids are known to use chemical cues (e.g. compounds produced by other Chironomidae larvae) and visual cues (e.g. vegetation cover) to detect favourable oviposition sites, such as newly flooded habitats where competition during larval

development would be minimal (Stevens et al., 2003). In their study, Stevens et al. (2003) do not give a direct answer as to why chironomids may avoid habitats with 2ppm of $NaNO_3$, although this may be inferred to be a result of the use of nitrate as a chemical cue. In the present study, Chironomidae abundance decreases with increasing nitrate concentrations in water (0.22-6.04 ppm nitrate), whereas EPT abundance increases. Adult female chironomids may avoid these sites for oviposition due to potential high competition with other invertebrates for the larvae. This would reduce larval recruitment in the stream stretch leading to the observed decrease in abundance.

Additional effects of nitrate on stream biota could relate to interactions with trace metals. Rijstenbil et al. (1998) observed that in adding copper (20ppb) to nitrate enriched media (2-3ppm nitrate) diatom cellular division was inhibited, whereas adding the same amount of copper in water without nitrate enrichment produced no effect on diatoms. Phytochelatines (PC) are peptides containing sulfhydryl amino acid cysteine with high affinity to copper and involved in the metal elimination mechanism in diatoms (Rijstenbil et al., 1998). Increases in copper concentration have been seen to increase PC pool in diatoms, thus PC concentrations in cells may be good metal pollution indicators. Rijstenbil et al. (1998) observed an increase of PC concentration and inhibition of cellular division in nitrate enriched environments (2-3ppm nitrate, 20ppb copper), compared to environments not enriched with nitrate but with the same copper concentration. This suggests that nitrate enrichment may affect copper exclusion and elimination mechanisms in diatoms. Similar interactions between nitrates and trace metals may occur in the study

streams, enhancing metal toxicity right at the bottom of the food web, thus affecting the whole community (Chapter 5).

In contrast to Gray (1998) and Malmqvist and Hoffsten (1999), zinc concentration in sediment or water does not seem to be the main factor affecting freshwater invertebrates in the present study. Gray (1998) observed a decrease in macroinvertebrate total abundance and richness with zinc concentrations of up to 640ppb. Malmqvist and Hoffsten (1999) studied streams polluted by metal mine drainage with zinc concentrations up to 1480ppb, reporting decreases in taxon richness but no change in abundance. Zinc concentrations in both studies are similar to those found in the present streams; however, they did not measure chemical species other than trace metals. Nitrate enrichment may not have been an issue in those streams, but an effect of nitrate on stream biota could have been undetected. Conversely, Hirst et al. (2002) considered a wider spectrum of dissolved solutes in the stream water (e.g. nitrate, trace metals and organic matter), and identified a negative effect of nitrate (average concentration 21ppm) and acidity (average value 6.7), and a positive effect of manganese (average concentration 62ppb) on macroinvertebrate species distribution. The effect of nitrate is not further discussed in their paper, but it is suggested that, when present in metal mine polluted streams, nitrate may have a negative effect on benthic communities, maybe by affecting chironomid oviposition as observed by Stevens et al. (2003), or exerting a synergic effect in conjunction with trace metals, as observed in enhanced copper toxicity for diatoms inhabiting nitrate rich environments (Rijstenbil et al., 1998).

4.6 Conclusion

Streams polluted by coal mine drainage are mainly characterised by an increase in conductivity, SS, iron concentrations in water and in sediment, and a decrease in pH (Table 4.1); whereas those contaminated by metal mine drainage are mainly characterised by high dissolved zinc in the water column and sediments loaded with zinc, lead and copper (Table 4.2). A significant increase in nitrate (from 1-2ppm to 2-3ppm) in the stream water has also been observed downstream from metal mine discharge, although this is not thought to be directly related to the mines but to grazing in the area (Chapter 3). This work suggests that coal and metal mine pollution exert different impacts on aquatic assemblages.

Coal mine pollution appears to have a severe impact on the invertebrate community structure, reducing species richness and abundance. The main factor operating here appears to be suspended sediments present in the water column, to which EPT are highly sensitive; whereas Chironomidae appear to be more tolerant. Seasonal differences have been observed in the response to pollution at the study sites: during summer and spring the impact of coal mine drainage appears to be reduced. This may be related to (a) Chironomidae (more tolerant to SS) dominance in warmer months, (b) favourable conditions in terms of food and temperature during summer (Jeyasingham and Ling, 2000), (c) life cycle and (d) faster development and reduced contact with pollution (Servia et al., 2004).

Metal mine pollution reduces species diversity and evenness at the study sites, but not abundance, suggesting a dominance of tolerant species. Furthermore, in these streams Chironomidae appear to be more sensitive than EPT. The environmental variables selected for the analysis explain only a small fraction of the species variation at the streams impacted by metal mine drainage, suggesting there might be other factors not accounted for that are influential. However, from the studied variables, nitrate concentration in stream water seems to exert a negative impact on the whole community but especially on Chironomidae. Although negative effects of nitrate on freshwater communities have been observed before in association with mine pollution (see Hirst et al., 2002), the reasons for this are not yet fully understood.

This study also suggests that sediment toxicity is not a good predictor of species distribution in streams impacted by mine drainage. Even though in coal mine drainage impacted streams sediment related variables appear to explain an important part of the species variation, this may be linked to the smothering effect of ochre flocs settling on the river bed more than a toxic effect of the trace metals associated with sediments. In the case of metal mine impacted streams, water chemistry appears to be a better predictor than sediment chemistry, probably due to the lack of extensive oxide precipitation.

5 ECOSYSTEM FUNCTION OF STREAMS IMPACTED BY MINE DRAINAGE

5.1 Introduction

In the previous chapter it has been proven that mine drainage impacts the structure of aquatic communities, however it has not been determined yet how this is translated into stream community function. Several studies discuss how mine pollution affects function from the point of view of organic matter decomposition (Forrow and Maltby, 2000, Nelson, 2000, Niyogi et al., 2001, Sridhar et al., 2001, Niyogi et al., 2002c, Carlisle and Clements, 2005) or primary production (Admiraal et al., 1999, Hill et al., 2000, Ivorra et al., 2002, Niyogi et al., 2002b), but a holistic approach including the functional structure of the aquatic community and studying the pathways of toxicity of different environmental variables is rarely considered.

5.1.1 Introduction to lotic function and mine pollution

The effect of mine pollution on organisms can be classified as direct and indirect. Direct effects include (a) the toxicity of heavy metals (Arnekleiv and Storset, 1995, Kelly, 1999, Bervoets and Blust, 2000), (b) pH effect on membrane permeability (Camargo et al., 2005), and (c) respiration difficulties linked to ochre smothering (Koryak et al., 1972, Hall et al., 1980). Whereas indirect effects are mainly related to (a) food availability and suitability (e.g. algal growth or detritus conditioning), (b) habitat modification (e.g. loss of habitat due to fine sediment (ochre) (Rabeni et al., 2005), and (c) interrelations with

other organisms (e.g. competitive or predator release). Generally, direct effects are easier to study and identify, however, indirect effects may be more significant in some environments (Fleeger et al., 2003). The conjunction of indirect effects mediated through consumer-resource interactions are often referred as 'trophic cascades' (Fleeger et al., 2003).

Different taxa have been seen to present varying sensitivities to mine pollution (Hickey and Clements, 1998, Malmqvist and Hoffsten, 1999, Richardson and Kiffney, 2000, Van Damme et al., 2008), which may be related to how the organisms are exposed to the pollution, e.g. trophic status and physical position in or on the streambed (Van Damme et al., 2008). Additionally, Hickey and Golding (2002) found that functional feeding groups were better predictors of mine pollution than structural measures.

Function measures how communities capture resources and convert these into biomass (Cardinale et al., 2006). It provides a holistic approach, integrating all direct effects and trophic cascades caused by pollution. Although the function of a community is thought to be linked to its species composition (i.e. community structure) (Chapin et al., 1997, Cardinale et al., 2006), this relationship has not always been identified (Hill et al., 2000), and other factors are also important, such as organism size and feeding rate (Forrow and Maltby, 2000). Therefore, it is important to study the ecosystem function as well as structure, integrating non lethal aspects and trophic cascades.

Functional classification used in this study follows Merrit and Cummins (1996), which differentiates groups according to feeding mechanisms not food type, as this may vary (Wallace and Webster, 1996). For example, grazers feed mainly on algae but may ingest other microorganisms while scraping, and filter-feeders ingest large quantities of

microorganisms suspended in the water column (Wallace and Webster, 1996). Additionally, some organisms have proven to be generalist feeders, fitting into more than one functional group. For example, Plecoptera, especially nemourids, apparently switch between algae and detritus as food sources depending on availability (Ledger and Hildrew, 2000). This behaviour seems to respond to competitive release when specialist grazers (such as mayflies) are absent in acidic streams (Ledger and Hildrew, 2001). A brief description of each guild's main food source is displayed in Table 5.1.

Table 5.1 Functional groups considered in this study and their main food sources

Functional feeding group	Food source
Collector-gatherers	FPOM[1] from stream bottom and microorganisms associated
Filter-feeders	Suspended FPOM from water column and microorganisms associated
Shredders	CPOM[2] and microorganisms attached to it
Grazers	Periphytic algae and associated material on mineral and organic substrates
Predators	Engulf whole body or suck body fluids of other animals

[1]FPOM: Fine particulate organic matter
[2]CPOM: Coarse particulate organic matter

In aquatic environments, the main sources of energy are allochthonous (e.g. leaf fall) and autotrophic (e.g. algal production); their proportion will vary depending on stream characteristics, such as shade and terrestrial organic matter input. Producers and decomposers are the principal drivers of matter and energy cycling within the ecosystem (Naem, 2001). However, the role of other groups such as collector-gatherers and filter-feeders should not be underestimated, as the retention of organic matter in the system strongly depends on them (Wallace and Webster, 1996).

5.1.1.1 Shredders and detritus decomposition

Leaf litter has a different chemical composition than the leaves on the tree. Before abscission, trees resorb most of the labile nutrients in green leaves (e.g. sugars and amino acids), leaving mainly cellulose, lignin and resistant carbohydrates, which are not digestible by most animals (Benfield, 1996, Moss, 1997). Thus, detritus entering the streams are nutritiously poor and need microbial conditioning to become palatable to animals. Once dead leaves enter a stream, many of the remaining soluble nutrients are leached into the water within 1 or 2 days (Benfield, 1996). Simultaneously, leaves are colonised by aquatic microorganisms, fungi seem to be the dominant group, whereas bacteria are more relevant in fine particulate organic matter (FPOM) conditioning (Cummins and Klug, 1979). Microorganisms digest the leaves using enzymes, and take up nitrogen and phosphorus from the water. This step is crucial for the litter conditioning, as colonised leaves, with fungal biomass rich in nitrogen, are a better food source than uncolonised leaves (Cummins and Klug, 1979, Moss, 1997).

In upland streams, coarse particulate organic matter (CPOM) is the principal food source (Wallace and Webster, 1996), hence, shredders play a critical role in the energy and matter cycles of these systems. They do not only feed on this matter, but convert CPOM into FPOM by physical action (i.e. little fragments detaching from CPOM during feeding), or via their faeces (Cummins, 1974). Hence, they represent a food source for other groups such as gatherers and filter-feeders (Cummins and Klug, 1979, Nelson, 2000). Although FPOM can also be formed mechanically due to current abrasion, shredder response to pollution represents a bottom-up as well as a top-down effect on the ecosystem.

Mine pollution impact on shredders and decomposition processes has been studied previously (Bermingham et al., 1996a, Nelson, 2000, Niyogi et al., 2001, Carlisle and Clements, 2005), but the toxicity pathways are still not well defined. Shredder sensitivity to zinc has been reported (Niyogi et al., 2001), whereas fungi appear resistant to this metal (Sridhar et al., 2001, Niyogi et al., 2002b, Duarte et al., 2008). Oxide deposition (common in streams polluted by coal mine drainage, Chapter 3) seems to impact on both microbes and shredders (Nelson, 2000, Niyogi et al., 2001, Sridhar et al., 2001). Hence,

changes in leaf litter decomposition can be due to a decrease in conditioning (Bermingham et al., 1996b, Forrow and Maltby, 2000, Nelson, 2000, Carlisle and Clements, 2005), direct effects on shredders (Niyogi et al., 2001, Sridhar et al., 2001, Carlisle and Clements, 2005), or more probably a combination of both.

Organic matter decomposition within an ecosystem such as a stream, is an integrative variable as it provides information at the ecosystem level, including microorganisms as well as macroorganism activities, and linking invertebrate trophic levels (Nelson, 2000). Hence, the study of leaf decomposition may provide information on partial trophic cascades, providing an assessment of the effect of mine drainage on the whole community

5.1.1.2 Grazers and periphyton

Biofilms are biological structures made of bacteria, algae, fungi and microfauna embedded in a mucopolysaccharidae matrix (Sabater et al., 2007). They are located at the bottom of the food web, and together with detritus, support most of the aquatic ecosystem, often forming the interface between water toxicants and the food web (Sabater et al., 2007). Hence they are a crucial factor to take into consideration when studying the function of any aquatic ecosystem.

Grazers are a key group in the energy and nutrient cycling of freshwater ecosystems, as they transfer the energy from primary producers (e.g. algae) up the trophic web. The top-down effect of grazers on the ecosystem is also important, as they often regulate periphyton growth (Hall et al., 1980, Wallace and Webster, 1996, Niyogi et al., 2002b, Gruner et al., 2008).

Similarly to other groups, grazer response to pollution does not only depend on water or sediment toxicity, but also on periphyton response. Literature is equivocal in describing the effect of acidity on algae, some studies observed negative effects (Maurice et al., 1987, Ledger and Hildrew, 2001, Verb and Vis, 2005), others only found a decrease in species richness but not abundance and suggested a decrease in food quality for grazers (Ledger and Hildrew, 1998, Ledger and Hildrew, 2005). Nevertheless, the negative effect

of acidity on specialised grazers (e.g. mayflies) appears to be widely accepted (Ledger and Hildrew, 2000).

Zinc contamination has also been found to negatively affect algal growth and grazers (Admiraal et al., 1999, Hill et al., 2000, Ivorra et al., 2002). However, Nyiogi et al. (2002) found a high biomass of algae but an absence of grazers in similar environments, and Admiraal et al. (1998) observed a shift towards zinc tolerant species in epilython.

Biofilms seem to be very sensitive to the physical aspect (oxide deposition) of mine pollution (Sridhar et al., 2001, Niyogi et al., 2002b). This has been related to (a) smothering of the algae (Niyogi et al., 2002b) (b) reduction of supporting substrata (Niyogi et al., 2002b), and (c) reduction of light penetrating the water column (Koryak et al., 1972, Ormerod et al., 1987, Niyogi et al., 2002b, Niyogi et al., 2002c).

5.1.1.3 Collector-gatherers and filter-feeders

Collector-gatherers and filter-feeders feed mainly on FPOM and are strongly related to shredder (Cummins and Klug, 1979, Wallace and Webster, 1996, Nelson, 2000), and grazer (Steinman and Lamberti, 1996) activity. Their main role in the ecosystem is retention of organic matter in the river segment, together with energy and mass transfer through the trophic web. A reduction in abundance of organisms belonging to these groups may result in a loss of organic matter with the flow (Wallace and Webster, 1996). They have also been found to increase organic matter particle size by ingesting FPOM and egesting larger compacted faecal particles (Cummins and Klug, 1979, Wallace and Webster, 1996). Hence they also represent a food source for other organisms.

The effect of mine drainage on gatherers and filter-feeders is rarely considered, despite the fact that they have proven to be better indicators of mine pollution than other guilds (Nelson, 2000).

5.2 Aims and objectives

This chapter studies the effect of coal and metal mine pollution on energy inputs (primary production and organic matter decomposition) and functional structure (collector-gatherers, filter-feeders, predators, grazers and shredders abundance and species richness) of the macroinvertebrate community in eight Welsh streams. The response pattern of organisms to environmental variables is not easily interpreted in most cases. This is due to many factors influencing their distribution other than direct toxicity, e.g. competitive, predator release, habitat modification, chemical and physical interactions between environmental variables (which may not always be additive). This project does not attempt to interpret specific relationships with explanatory variables as this is not possible in field studies, the main aim is to determine which factors of mine pollution affect the organisms to provide information that can contribute to the improvement of treatment schemes.

The main objectives are to:

a) Determine if abandoned mine drainage negatively affects the function of the aquatic community in receiving streams (energy inputs and functional structure);

b) Detect differences in ecosystem functional response to coal and metal mine drainage pollution;

c) Relate environmental variables to functional changes in the benthic community.

5.3 Methodology

5.3.1 Ecosystem function analysis

Ecosystem function in the study streams was analysed from two points of view: (1) main energy inputs in the stream (production and decomposition) and (2) functional structure.

5.3.1.1 Decomposition measurements

Oak (*Quercus robur*) leaves were collected from trees just before ready to fall, air dried in cardboard boxes and stored in dry conditions for a year.

Approximately 5 grams of dry leaves were placed in mesh bags and the specific weight noted. Two different mesh sizes were used in order to differentiate between microorganism and macroinvertebrate activity: 250μm and 0.5cm (Benfield, 1996).

Four leaf packs of each type were placed in each sampling site (upstream and downstream from the polluting mines, Figures 2.1-2.9, Chapter 2) for 15 weeks over the summer of 2007 (Plate 5.1). After this period of time, leaf packs were removed from the water, placed in individual plastic bags and kept cool during transport to the laboratory before being frozen until processing.

Leaves were removed from the mesh bag in the lab and gently rinsed of silt and debris. Subsequently, leaves were placed in paper bags and left to dry until constant weight before weighing.

Extra leaf packs were prepared to evaluate “handling loss”, since dry leaves are easily broken. These bags were submitted to the entire process (except stream incubation). The difference between their initial and final weight has been considered negligible (1% loss in coarse mesh bags, 2% loss in fine mesh bags). Hence, the difference between initial dry mass and final dry mass corresponds to the amount of leaves decomposed during the experimental period, this has been expressed as a percentage of the initial mass. Shredder decomposition has been isolated from microbial activity by subtracting fine mesh mass loss (microbes) to coarse mesh mass loss (total=microbes+shredders).

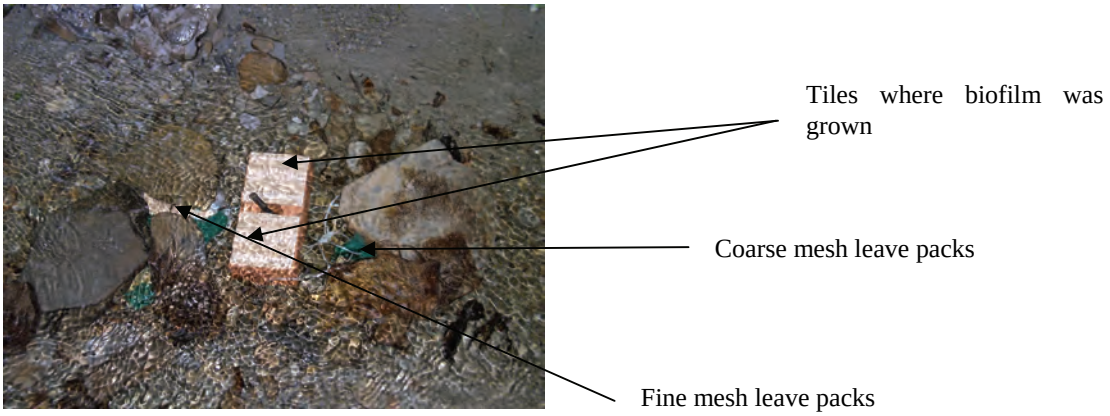


Plate 5.1 Brick holding 2 tiles where biofilm was grown and 4 leaf packs (2 coarse mesh and 2 fine mesh) (Source: Original)

5.3.1.2 Production measurements

Artificial substrata have been used to grow biofilm because this is crucial for standardisation of results (Sabater et al., 2007). Six tiles were placed in each sampling point (Figures 2.1-2.9, Chapter 2) to be colonised by algae (Plate 5.1). After 15 weeks of exposure, tiles were removed from the water and the biofilm was brushed into a vial. The slurry collected (24ml) was kept in ice on the dark until frozen in the laboratory (Steinman and Lamberti, 1996).

The method of Ledger and Hildrew (1998) was followed to measure chlorophyll content in the stream biofilms. 10ml of the sludge previously frozen was freeze-dried in order to eliminate the water from the sample losing as little algae as possible. This method seems to facilitate chlorophyll extraction as it breaks the cell walls (Ledger, pers. comm.). The chlorophyll was extracted from the sample overnight at 5ºC in 90% acetone. Acetone dilutions were centrifuged prior to analysis because this was seen to reduce turbidity readings in the sample. Spectophotometric measurements were taken at 664, 647, 630 and 750nm. Absorbance at 750nm was used to correct results for turbidity by subtracting it from the absorbance at the other wave lengths.

The following equations were used for the calculations (Sterman, 1988).

$$\text{Chlorophyll a} = 11.85A_{664} - 1.54A_{647} - 0.08A_{630}$$

$$\text{Chlorophyll b} = -5.43A_{664} + 21.03A_{647} - 2.66A_{630}$$

$$\text{Chlorophyll c} = -1.67A_{664} - 7.6A_{647} + 24.53A_{630}$$

Where A represents the absorbance readings at each wavelength.

5.3.1.3 Functional structure

All macroinvertebrates identified in the study sites were classified into functional groups (see section 2.4.2 in Chapter 2). Table 5.1 shows the considered groups for this study and a brief description of their food sources, dietary generalists have been classified into one group following the literature.

Ephemeroptera, Plecoptera and Trichoptera were classified following Moog (1995). Merrit and Cummins (1996) was used to classify Chironomidae, complemented by information from Wilson and Ruse (2005).

Abundance and species richness was calculated for each functional group on each site using Species Diversity and Richness II program from Pisces Conservation Ltd.

5.3.2 Environmental variables

In this study, abundances of functional groups were related to several environmental variables linked to mine pollution. Only those variables that significantly vary downstream from the mine and had not correlation with other variables were considered for this analysis (Chapter 3). DO is the only variable that significantly decreases downstream from coal mine discharges but has not been included in the analysis, since it was always around saturation (Appendix A, Tables 3.5 and 3.6, Chapter 3), thus it is not thought to represent a limiting factor for macroinvertebrates.

Each type of mine pollution studied causes different effects on the water and sediment chemistry of the recipient streams. Streams impacted by coal mine drainage were characterized by: increase in sulphates, dissolved iron, manganese, suspended solids (SS),

total dissolved solids (TDS), conductivity (EC), cations (sodium, potassium, magnesium and calcium), and iron concentration in sediments; and a decrease in pH (Table 4.1 and Chapter 3). Streams polluted by metal mine drainage were characterised by an increase in sulphate, conductivity, TDS, dissolved zinc, nitrate, chloride, and zinc, lead and copper concentrations in sediments (Table 4.2 and Chapter 3).

Due to the common origin of these variables and what they represent, they are highly correlated. GAM cannot be used when there is co-linearity between explanatory variables, as the results obtained from the analysis may be invalid (Zuur et al., 2007), thus variables with correlation coefficient higher than 0.50 were discarded from the analysis (Chapter 3). Table 5.2 shows the variables used for the analysis plus those they represent in coal mine impacted sites, and Table 5.3 in metal mine polluted streams.

Table 5.2 Environmental variables in streams impacted by coal mine drainage

Variable used	Correlated variables
Iron in water	Manganese in water
SS	Sulphate, TDS, EC, calcium, potassium, magnesium and sodium
pH	None
Iron in sediment	None

Table 5.3 Environmental variables in streams impacted by metal mine drainage

Variable used	Correlated variables
Zinc in water	None
EC	Sulphate, total dissolved solids and chloride
Nitrate	None
Zinc in sediment	Copper and lead in sediment

5.3.3 Statistical analysis

Several standardised measures were available for each sampling site (6 chlorophyll contents readings, 4 shredder and 4 microbial decomposition measures). One-way ANOVA was used to test for differences between populations upstream and downstream of the main discharges at each study site.

Wilcoxon signed rank test was used to analyse for differences in functional diversity in the streams. In order to identify differences caused by mine drainage in each ecosystem avoiding interferences by differences between streams, it was considered necessary to use a paired test. Non-parametric statistics were used due to lack of normality in the data.

Forward selection in GAM (Poisson distribution with log link and 4df) was used to identify those variables that best explained the variance in the functional groups abundance using Brodgar 2.5.6. This test was chosen because non-linear models seem to fit better with the ecological data variation and the homogeneity assumption was not fulfilled (Section 2.5.2.2, Chapter 2). Models with a significant ($p<0.05$) output were considered in the analysis. Functional group abundance data was in the form of a percentage to provide a better model fit.

The same program was used to draw figures that allow visualisation of the response pattern of functional groups to the model selected by GAM. When more than one variable was chosen by GAM, the first variable of the model was considered to be the most representative and was used to draw the figures.

Correlations between different guilds were calculated and presented in pair graphs drawn with Brodgar 2.5.6.

5.4 Results

5.4.1 Energy inputs in coal mine drainage impacted streams

To monitor energy inputs into the streams, leaf litter decomposition and algal production were studied. Figure 5.1 demonstrates that litter processing decreases downstream from the studied coal mines. Nevertheless, only the differences amongst the coarse mesh bags (total decomposition) are statistically significant ($F=16.115$, $p<0.01$).

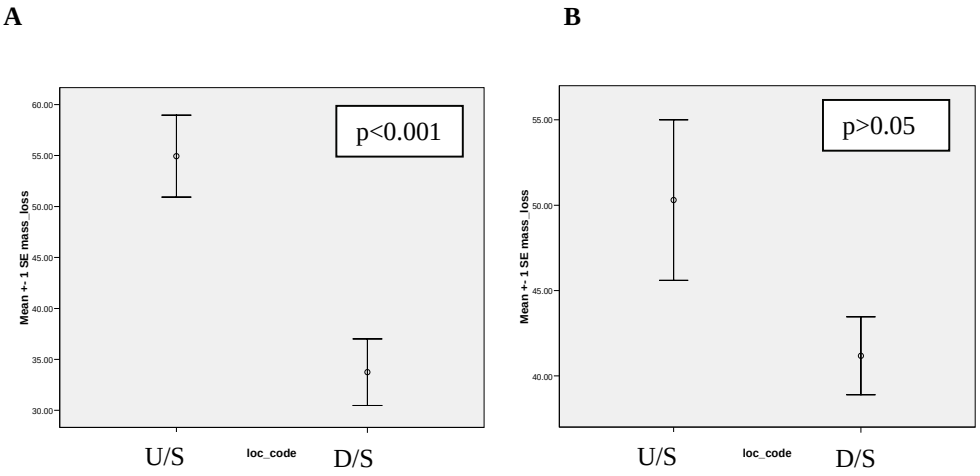


Figure 5.1 Mean litter decomposition (% mass loss) (± 1SE, n=32) upstream and downstream from coal mines in summer 2007. (A) Coarse mesh leaf packs, (B) fine mesh leaf packs

Table 5.4 depicts the differences between microbial (fine mesh bags) and non-microbial (difference between coarse and fine mesh bags) decomposition in streams impacted by coal mine drainage. Shredder (non-microbial) decomposition appears to be the only fraction decreasing downstream from the mine discharge ($p=0.036$). However, the majority of detritus decomposition appears to be undertaken by microbes.

Table 5.4 Mean microbial and non-microbial litter decomposition (% mass loss, n=32) upstream and downstream from coal mine drainage

	Microbial decomposition (% mass loss)	Non-microbial decomposition (% mass loss)
Upstream	47	11
Downstream	41	2
p	>0.05	0.036

Total chlorophyll content was measured in biofilms collected in streams impacted by coal mine drainage to monitor primary production in these systems. Unfortunately, the orange colouration of the solutions caused by ochre interfered with the method used, giving erroneous values. Hence, these data are not available. However, visual differences were found (Plate 5.2): samples collected downstream from the mines were orange, whereas those collected upstream were green.

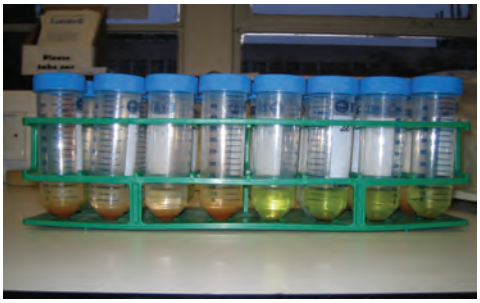


Plate 5.2 Vials holding biofilms from coal mine drainage impacted streams after acetone extraction. Four vials on the left are downstream samples, four on the right upstream (Source: Original)

5.4.2 Functional structure in coal mine drainage impacted streams

Table 5.5 depicts the effect of coal mine drainage on functional structure. Species number of all guilds, except filter-feeders (Z=-0.114, p>0.05), significantly decrease downstream from the coal mines. In terms of abundance, only grazers (Z=-2.806, p=0.005) and predators (Z=2.488, p=0.013) are significantly affected by this pollution.

Table 5.6 shows that collector-gatherers and predators seem to be negatively affected by pH in the stream water (p<0.001) (Figure 5.2). Grazer abundance increases with SS concentration (p<0.001) (Figure 5.2).This pattern appears to be dominated by the increase of species not belonging to Ephemeroptera, Plecoptera, Trichoptera or Chironomidae (EPTC), but mainly to invertebrates belonging to Gastropoda and Elminthidae (Figure 5.4). Shredders are negatively affected by increased iron concentration in sediments (p<0.001) (Figure 5.3). Filter-feeders suffer a clear negative impact with increasing iron concentration in water (p<0.001) (Figure 5.2).

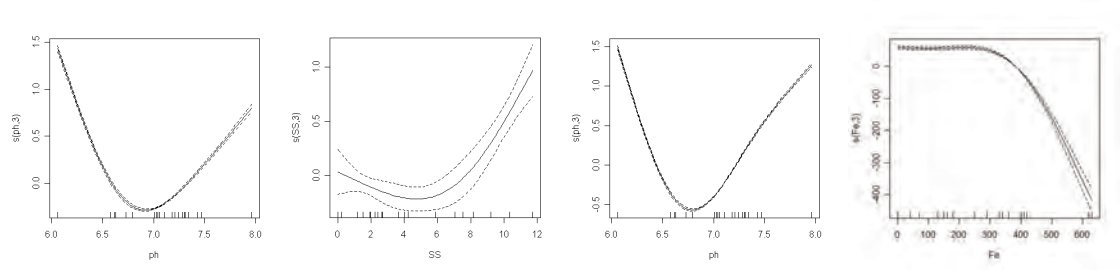
Table 5.5 Variation in species number and abundance of functional groups (n=32) upstream and downstream from coal mines according to Wilcoxon signed rank test between summer 2006 and spring 2007

	Species No		Abundance	
	Z	p	Z	p
CG	-2.556	p=0.011	-1.244	p>0.05
GR	-3.181	p=0.001	-2.806	p=0.005
SH	-2.272	p=0.023	-1.191	p>0.05
PR	-2.983	p=0.003	-2.488	p=0.013
FF	-0.114	p>0.05	-1.922	p=0.055

Table 5.6 Model explaining functional group relative abundance distribution in streams impacted by coal mine drainage between summer 2006 and spring 2007 according to GAM (n=32)

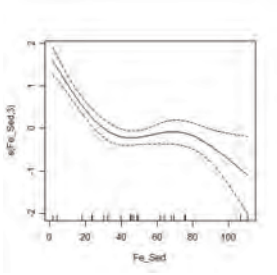
Guild	Model	p
CG	pH	p<0.001
GR	SS	p<0.001
SH	Iron in sediments, TSS, iron, pH	p<0.001
PR	pH	p<0.001
FF	Iron	p<0.001

CG: Collector-gatherers
GR: Grazers
SH: Shedders
PR: Predators
FF: Filter-feeders



Collector-gatherers Grazers Predators Filter-feeders

Figure 5.2 Variation in relative abundance of collector-gatherers, grazers, predators and filter feeders explained by the models displayed in Table 5.5



Shredders

Figure 5.3 Shredder relative abundance response to iron concentration in sediments, following the model displayed in Table 5.5

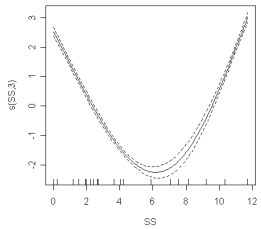


Figure 5.4 Grazer relative abundance response to SS considering only those organisms not belonging to EPTC according to GAM with Poisson log link distribution

Figure 5.5 depicts very high correlation between collector-gatherers and predators (r=0.84). Relationships between other guilds seem much weaker, as their correlations are very low.

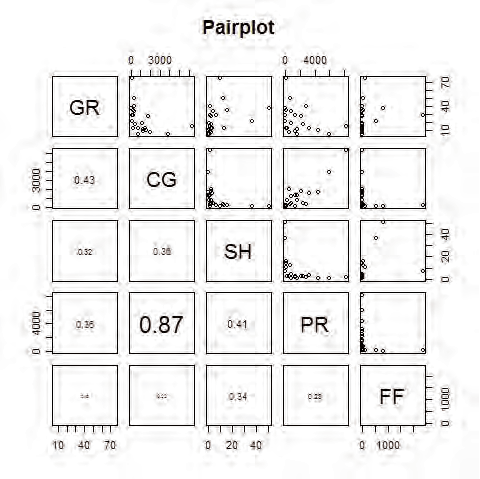


Figure 5.5 Correlations between functional groups in coal mine impacted streams

5.4.3 Energy inputs in metal mine drainage impacted streams

Following Nyiogi et al. (2001) who suggest a zinc concentration threshold above which shredder activity is inhibited (500ppb), decomposition results at metal mine sites were separated according to their zinc concentrations. M1 represents metal mine sites with zinc concentrations higher than 500ppb (Frongoch and Dilyfe mines), whereas M2 represents those with lower zinc concentrations (<500ppb) (Cwm Symlog and Cwm Ystwyth mines).

Figure 5.6 demonstrates that metal mine pollution severely impacts on total detritus decomposition ($F=36.207$, $p<0.001$) and microbial decomposition ($F=7.574$, $p=0.016$) when zinc concentrations exceed 500ppb. On the other hand, in lower zinc concentrations total decomposition does not seem to be significantly affected ($F=0.729$, $p>0.5$) and microbial activity seems to be slightly stimulated ($F=4.922$, $p=0.047$) (Figure 5.7).

A B

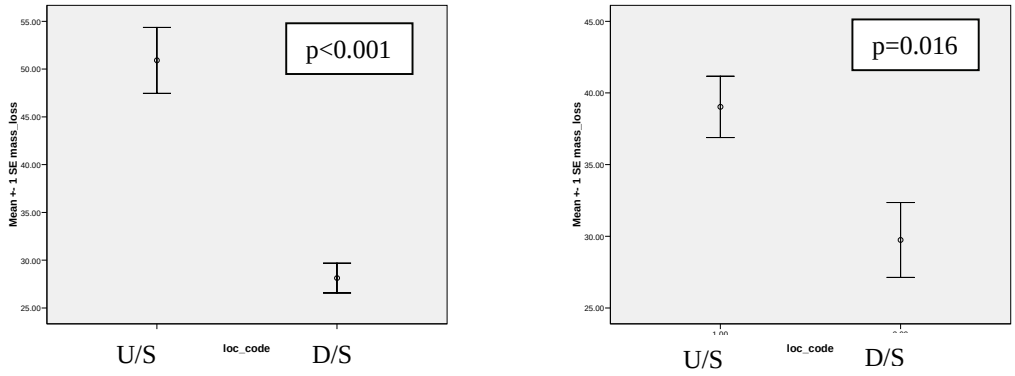


Figure 5.6 Mean litter decomposition (% mass loss) (± 1SE, n=16) upstream and downstream from 2 streams impacted by metal mine drainage (Zn>500ppb) in summer 2007. (A) Coarse mesh leaf packs, (B) fine mesh leaf packs

A **B**

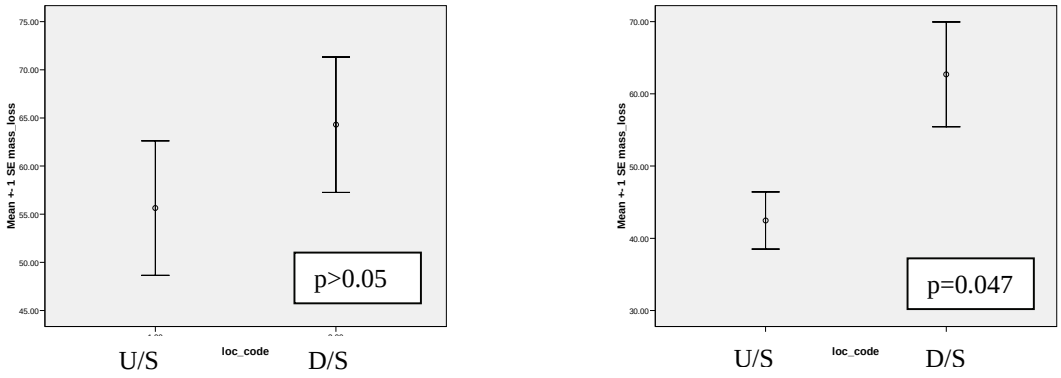


Figure 5.7 Mean litter decomposition (% mass loss) (± 1SE, n=16) upstream and downstream from 2 streams impacted by metal mine drainage (Zn<500ppb) in summer 2007. (A) coarse mesh leaf packs, (B) fine mesh leaf packs

Table 5.7 depicts the differences between microbial (fine mesh) and non-microbial (coarse mines minus fine mesh) decomposition in both metal mine groups (M1 and M2). Similarly to coal mine impacted streams, shredder decomposition follows similar patterns than total decomposition, decreasing downstream from M1 ($p=0.015$) but not M2 ($p>0.05$). Additionally, microbial activity also appears to be greater than non-microbial decomposition.

Table 5.7 Mean microbial and non-microbial litter decomposition (% mass loss, n=16) upstream and downstream from two groups of streams polluted by metal mine drainage, M1 (zinc concentration greater than 500ppb) and M2 (zinc concentration lower than 500ppb)

		Microbial decomposition (% mass loss)	Non-microbial decomposition (% mass loss)
M1	Upstream	39	13
	Downstream	29	2
	p	0.016	0.015
M2	Upstream	43	13
	Downstream	63	13
	p	0.047	>0.05

Figure 5.8 demonstrates a negative impact of metal mine drainage on algal production (total chlorophyll) ($F=4.370$, $p=0.018$).

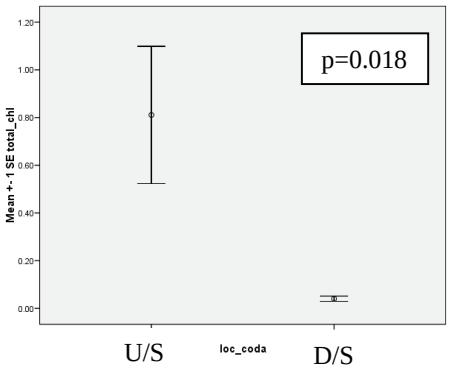


Figure 5.8 Mean total chlorophyll (µg/ml) (±1 SE, n=48) from biofilms collected in streams impacted by metal mine drainage in summer 2007

5.4.4 Functional structure in metal mine drainage impacted streams

Only collector-gatherer species number significantly decreases downstream from metal mine drainage discharge (Z=-2.446, p=0.014), whereas all the other guilds do not seem to be impacted by this type of pollution (Table 5.8).

Nevertheless, GAM (Poisson distribution with log link and 4df) results demonstrate significant patterns between each functional group and the environmental variables chosen for the analysis (Table 5.9). Zinc concentration in water seems to be a critical factor affecting collector-gatherer ($p<0.001$), shredder ($p<0.001$) and filter-feeder ($p<0.001$) abundance (Table 5.9). Filter-feeders present a clear reduction in abundance as zinc concentration in water increases (Figure 5.10); whereas collector-gatherer response pattern to zinc concentrations in stream water is rather complicated, with consecutive increases and decreases (Figure 5.9).

Predator response to metal mine pollution is related to zinc concentration in sediments ($p<0.001$), with an initial decrease in abundance followed by an increase (Figure 5.10). Grazer abundance initially increases with nitrate concentration, but the overall effect of this nutrient on the guild is negative ($p<0.001$) (Figure 5.9).

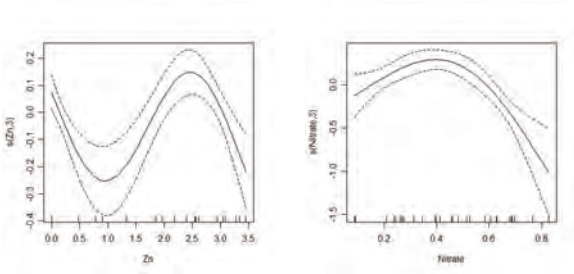
Table 5.8 Variation in functional group species number and abundance (n=32) upstream and downstream from metal mines according to Wilcoxon signed rank test

	SpNo		Abundance	
	Z	p	Z	p
CG	-2.446	p=0.014	-1.396	p>0.05
GR	-0.915	p>0.05	-0.768	p>0.05
SH	-1.201	p>0.05	-0.768	p>0.05
PR	-1.375	p>0.05	-1.552	p>0.05
FF	-0.979	p>0.05	-0.931	p>0.05

Table 5.9 Significant variables explaining functional group relative abundance (n=32) distribution in streams impacted by metal mine drainage according to GAM

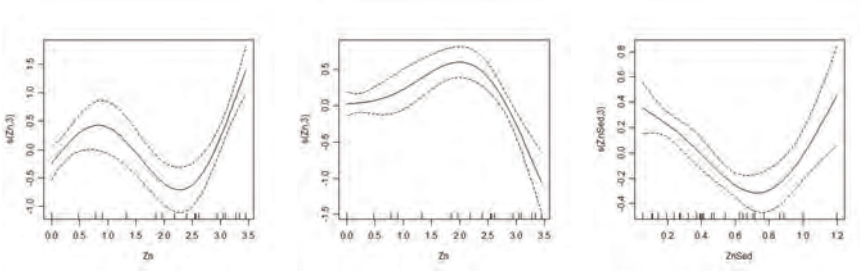
Group	Model	p
CG	Zinc	p<0.001
GR	Nitrate	p<0.001
SH	Zinc, nitrate, EC, zinc in sediments	p<0.001
PR	Zinc in sediments	p<0.001
FF	Zinc, nitrate, zinc in sediments, EC	p<0.001

CG: Collector-gatherers
GR: Grazers
SH: Shedders
PR: Predators
FF: Filter-feeders



Collector-gatherers Grazers

Figure 5.9 Collector-gatherer and grazer abundance variation explained by the model displayed in Table 5.7



Shredders Filter-feeders Predators

Figure 5.10 Shedder, filter-feeder and predator abundance variation explained by the first variable in the model displayed in Table 5.7

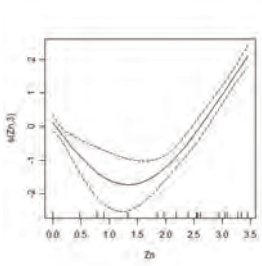


Figure 5.11 Shredder abundance variation with dissolved zinc considering only Chironomidae according to GAM

Collector-gatherers seem to be correlated with most of the guilds in the studied ecosystems, as their correlation coefficients are quite high with grazers (r=0.75), predators (r=0.60) and filter-feeders (r=0.56).

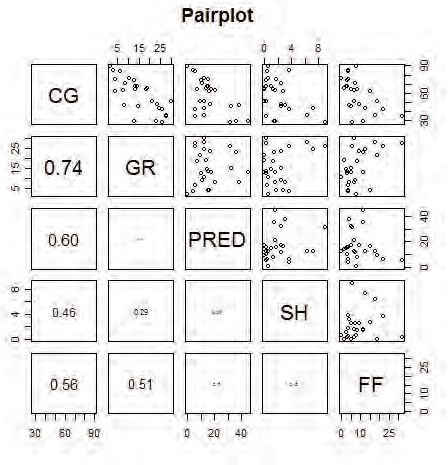


Figure 5.12 Correlations between functional groups in metal mine polluted streams

5.5 Discussion

5.5.1 Function in coal mine drainage impacted streams

Total decomposition is clearly reduced downstream from the studied coal mines. Microbial activity (47% mass loss upstream and 41% mass loss downstream) appears to be dominant in these environments, especially downstream from the mines, suggesting that shredder activity is very limited (11% mass loss upstream and 2% mass loss downstream). From both groups, shredders appear to be the most impacted by coal mine pollution, and the reasons for this are explored below.

Although shredder abundance does not seem to decrease at the downstream sites, the number of species forming this group does, suggesting that species diversity affects shredder feeding activity, which was also found by Jonsson et al. (2001). Litter breakdown may decrease with shredder species richness because intra-specific competition or interference is higher than inter-specific, or because inter-specific facilitation may favour litter processing where more species are present (Jonsson and Malmqvist, 2000, Jonsson et al., 2001, Jonsson et al., 2002).

Further analysis of shredder abundance indicates that sediment contamination may be a determining factor when describing its pattern. Iron concentration in sediment can be seen as an indicator of ochre deposition on the bed of the streams, meaning that shedder abundance is affected by ochre precipitation. This could be due to (a) a direct effect on organisms such as clogging of their gills (Koryak et al., 1972, Hall et al., 1980) (Plate 5.3) or toxicity via ingestion of sediments during feeding, or (b) indirect effects related to

food availability and suitability. Ochre deposition may coat the leaves acting as a protective layer and preventing access (Nelson, 2000), or microbial conditioning may be affected due to the pollution. The latter is unlikely at the study sites, as microbial decomposition of leaf litter during the experiment was not significantly reduced.

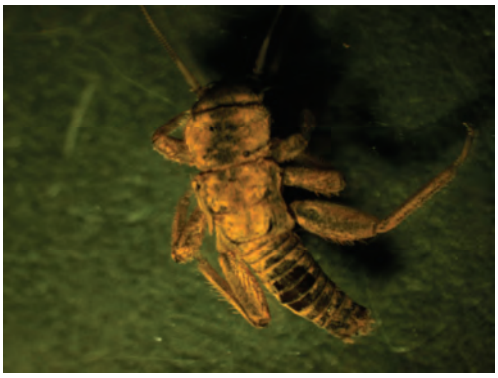


Plate 5.3 *Protonemura praecox*. Shredder from a site polluted by coal mine drainage covered with ochre (Source: Original)

Grazers seem to be a group especially affected by coal mine drainage, as both abundance and species number are significantly reduced downstream from the mines. Unfortunately, quantitative evidence of reduction of food source (periphytic algae) is not available for the study sites. However, algal growth has been reported to decrease in other streams impacted by coal mine drainage (Sridhar et al., 2001, Niyogi et al., 2002b). This could be due to ochre smothering of algae (Niyogi et al., 2002a), lack of substratum available for growth (Niyogi et al., 2002a), or reduction of light due to increased turbidity of the water (Koryak et al., 1972, Ormerod et al., 1987, Niyogi et al., 2002b). Field observations indicate that a reduction in algal growth might be occurring at the study sites, as ochre covers the stream bed downstream from the coal mines.

However, GAM results suggest an increase in grazers with SS (Figure 5.2), which is at odds with the possible reduction in algal biomass in the study streams. Grazer response pattern to SS is similar to that followed exclusively by grazer macroinvertebrates not belonging to EPTC recorded at the site, mainly Gastropoda and Elminthidae. These may present tolerance to ochre smothering, and therefore would thrive due to competitive or predator release. EPTC follow a different pattern, with a reduction of organism abundance at high SS concentrations. It must be taken into account that SS also represent the electrolyte characteristics of the stream water. Hence the response pattern of grazers is difficult to interpret with the available data.

Collector-gatherer species number is significantly reduced downstream from the mines, yet not their abundance, suggesting that tolerant species may thrive in these environments. Figure 5.2 shows how this guild abundance increases at lower (<6.5) and higher (>7.5) pH, probably due to competitive release, leading to tolerant species thriving at both intervals.

Predators seem to be highly affected by coal mine drainage, as both abundance and species number are significantly reduced downstream from the mines. Even though freshwater invertebrate predators are generally polyphagus (feed on a variety of prey types) (Giller and Malmqvist, 2008), gatherers seem to be the main prey for this guild at the study sites, as they are highly correlated and respond similarly to pH. Thus, predator response to pH may follow food availability in form of gatherer prey.

Even though filter-feeder abundance or species number do not seem to be reduced by coal mine pollution when examining upstream and downstream differences, GAM results show a significant effect of iron and manganese concentration on their abundance.

Because filter-feeders obtain their food directly from the water column, it is likely that their distribution will be influenced by water contamination. Trace metal effect on organisms has been discussed in Chapter 4, and is mainly related to biochemical reactions, generally manifested as hypoxia (deficiency of oxygen reaching the body tissues), caused by a reduction in gas exchange due to coagulation and precipitation of mucus or cytological damage (Koryak et al., 1972, Sridhar et al., 2001, Niyogi et al., 2002a).

5.5.2 Function in metal mine drainage impacted streams

Niyogi et al. (2001) suggest a threshold of 500ppb of zinc above which shredder activity is inhibited. In the present study, the same pattern is observed, as M1 (>500ppb of zinc in water) show a significant decrease of litter breakdown, whereas M2 (<500 ppb Zn) does not. Similarly to coal mine impacted streams, microbial decomposition appears to be dominant in streams impacted by metal mine discharge, especially downstream from the most polluting mines (M1, Zn>500 ppb) (29% mass loss due to microbes and 2% by shredders). The role of shredders and microbes in litter decomposition is discussed below.

Microbial activity in M2 (Zn<500 ppb) seems to be slightly stimulated. This resilience of the ecosystem at low zinc concentrations has been linked to fungal tolerance to the metal (Nelson, 2000, Niyogi et al., 2001, Duarte et al., 2008), which might be stimulated at low concentrations due to suppression of competition or predation, or favourable environmental conditions (Niyogi et al., 2002).

The inhibition of microbial activity found in M1 might suggest that there is also a threshold between 280ppb (highest concentration recorded downstream in M2 during summer 2006 when the experiments took place) and 700ppb (lowest concentration recorded downstream in M1 during summer 2006), above which microbial processing is affected. The literature is equivocal in establishing a threshold for zinc concentration above which microbial activity is affected. Duarte et al. (2004) observed a reduction in fungal activity in laboratory experiments at concentrations above 33ppb (much lower than 700ppb), whereas Nyiogi et al. (2001) did not notice any effect in streams with zinc concentrations up to 80ppm.

Considering shredder abundance and species number, no significant changes seem to occur downstream from the mines, even when sites are classified into M1 and M2 groups. This might suggest that other variables not reflected in the structure of shredder assemblage may be affecting their activity, e.g. a reduction in feeding rate (Forrow and Maltby, 2000). This is further discussed below.

Shredder abundance presents a significant response to zinc concentration in water. The pattern of shredder abundance along the zinc gradient is rather complicated. The initial increase (<10ppb Zn) in shedders could be a response to an initial stimulation of CPOM conditioning by fungi, making more palatable food readily available. This is followed by

a decrease in shredder abundance (10 to 250ppb Zn) possibly due to water toxicity or an effect on mycoflora reducing detritus conditioning. The final increase in shredder abundance (>250ppb Zn) is the most contradictory part of the response, as shredder decomposition is clearly reduced in streams presenting higher zinc concentrations. Nevertheless, this could be due to tolerant taxa thriving due to competitive and/or predator release, such as Chironomidae which show an increase in shredder abundance with increasing zinc concentration. Forrow and Maltby (2000) suggest that an increase in abundance is not always linked to an increase in feeding activity. Tolerant species that replace more sensitive species can present different feeding rates, or these might be affected by the pollution, ending with an overall decrease in decomposition. Additionally, intra-specific and inter-specific interactions depend on species identity (Jonsson et al., 2002), hence a variation in species present in the ecosystem may lead to modification of litter processing rates.

Algal growth has been reported to be negatively affected by zinc pollution in some studies (Hill et al., 2000, Ivorra et al., 2002), although other studies found algae abundance to increase with zinc concentrations up to 80ppm, possibly due to lack of grazers in those waters (Niyogi et al., 2002b), or did not detect any change at all (Admiraal et al., 1999). The present study demonstrates a clear reduction on algal biomass downstream from the metal mines.

A decrease in algal biomass would be expected to be followed by a decrease in grazers, as their food source has been diminished. Nevertheless, upstream and downstream comparisons do not show this occurring. GAM results suggest that grazer abundance decreases as nitrate concentration in water increases (2-3ppm). This negative response to

an increase in nutrients contradicts previous work studying this relationship with similar nitrate concentrations (Camargo et al., 2005), which proved that nutrient enrichment translates into higher algal biomass and hence more food available for grazing. However, in the study streams zinc toxicity may inhibit positive effects by nutrient enrichment on primary production.

In Chapter 4 it has already been discussed the role of nitrate in conditioning macroinvertebrate species distribution at the study sites. Nitrate appears to exert a negative effect on Chironomidae, which may be related to decrease in oviposition as observed in *Chironomus teppori* at similar nitrate concentrations (2ppm) (Stevens et al., 2003). Additionally, Rijstenbil et al. (1998) found that nitrate (2-3ppm) enhances copper toxicity to diatoms. Hence, nitrate could be interacting with metals present in the stream water impacting on primary production, this could then be transported up the food chain, affecting grazers.

Collector-gatherers seem to be the only group where significant differences upstream and downstream from the metal mines are found, as their species number decreases significantly. GAM results show that their distribution is significantly influenced by zinc concentration in water. As observed for shredders, the response in collector-gatherer populations was found to be complicated. The initial increase in abundance (between 10 and 316ppb of zinc) may be due to competitive or predator release, but as zinc concentration increases (>316 ppb Zn), their abundance is clearly reduced. Nelson (2000) also reported a negative effect of zinc pollution on collector-gatherers. This could be due to two processes (a) direct toxicity of zinc on the organisms or (b) indirect effect via food availability. Effects of trace metals, such as zinc, on biochemical reactions at cellular

level in macroinvertebrates has been discussed in Chapter 4, here the indirect effect via food availability will be discussed.

Collector-gatherers feed mainly on FPOM. FPOM is mainly formed from CPOM during shredder feeding or by mechanical abrasion (Cummins and Klug, 1979, Nelson, 2000) and from biofilms during grazer feeding (Steinman and Lamberti, 1996). If shredder and grazer activity decreases, this could result in less FPOM production. Alternatively, bacteria are the main group responsible for FPOM conditioning (Cummins and Klug, 1979), and these have been shown to be affected by zinc pollution (Admiraal et al., 1999, Carlisle and Clements, 2005). Moreover, Nelson (2000) suggests that algae growing on FPOM may contribute to its conditioning, or be a direct source of food for collector-gatherers, hence a decrease in algae probably due to metal toxicity may reduce food availability for this group as well. In summary, production of FPOM could be inhibited and conditioning of detrital material reduced, resulting in a dearth of suitable food available for collector-gatherers.

Even though filter-feeder abundance and species number does not vary significantly downstream from the metal mines, their distribution is significantly related to the pollution. Zinc concentration in water represents a clear toxic agent for this guild as seen in Figure 5.10. Similarly to collector-gatherers there might be two reasons for this negative effect (a) direct toxicity of the metal on these organisms, and (b) indirect effect through lack of quality/quantity of food. Since filter-feeders also feed on FPOM, the causes discussed for collector-gatherers are also applicable.

Predators do not seem to be affected by metal mine drainage in terms of upstream and downstream differences in abundance and species number. But their abundance seems to

be linked to sediment toxicity. Direct effect of heavy metal bound to sediments on predators is unlikely, as they do not feed from the sediments and being mobile may be able to move from unsuitable sediments (Rabeni et al., 2005). Hence, the effects on predators may be due to effects on prey (e.g. collector-gatherers, as they have a high correlation index) and competition.

5.6 Conclusion

The energy and matter flow in ecosystems polluted by mine drainage is clearly impoverished, as decomposition and algal growth are both affected, impacting on the cycle from the bottom of the food web. Even though macroinvertebrate abundance was not always significantly reduced downstream from the mines, other variables not directly related to the structure may be affected (e.g. feeding rate and species richness), resulting in an impoverished ecosystem. This demonstrates the need to include functional analysis when studying how pollution impacts on receiving ecosystems, as this provides a holistic approach including more factors than just ecosystem structure.

The main aim of this study was to relate ecosystem function to the environment. This analysis showed that different functional groups respond to different aspects of the pollution. At the same time, different mine types seemed to cause a different effect on the community.

Organisms living in streams impacted by coal mine drainage, typically accompanied by extensive ochre precipitation and a decrease in pH (from a maximum of 7.4 to a minimum of 6.7), tend to respond to a mixture of these environmental stressors. Grazers and shredders respond to a more physical aspect of the pollution related to ochre deposition, namely SS and iron concentration in sediments. Predator and collector-gatherer distribution can be explained by pH variation in the stream water, whereas filter-feeders seem to be mainly affected by dissolved iron.

On the other hand, invertebrates living in streams polluted by metal mine drainage (these study sites did not present extensive oxide precipitation) tend to respond to chemical aspects of the pollution. Shredders, filter-feeders and collector-gatherers are linked mainly to zinc concentration in water, and predators respond to zinc concentration in sediment. The effect on grazers seems to be masked by an increase in nitrate concentration in the stream water, which is not thought to be linked to mine pollution but to agriculture in the area. The negative impact of nitrate in grazers and in the whole community (Chapter 4) is not well understood, but it might be related to enhanced metal toxicity on primary production or negative effect on Chironomidae oviposition.

It is difficult to distinguish between direct and indirect (e.g. suitable food and interactions with competitors) effects of pollution on organisms. For example, shedders are closely linked to microbial activity in streams, as organic matter palatability will depend on them. Therefore, a reduction in shredder activity may be due to a response to a reduction in suitable food availability, pollution toxicity or physical effect on the organisms. Field studies like the present one are not able to discern between these direct and indirect effects, for this purpose further study involving controlled experiments would be required.

Conclusions drawn from Chapter 4 suggested that sediment quality was not a good predictor of mine impact on freshwater communities, however sediments have proven to impact on functional responses to mine pollution. Therefore, sediment analysis should be included in routine monitoring of freshwater environments, as it complements water

quality in explaining and detecting pollution effects on the function of aquatic ecosystems.

The present study provides important information to consider when planning mine drainage remediation. Coal mine drainage remediation needs to focus on acidity and ochre precipitation in order to improve the function of the receiving streams. These are the main aspects already tackled by engineered wetlands, since the settling ponds and aerobic wetlands have shown to be effective at oxygenating the water causing ochre to precipitate and pH to rise to circumneutral values (Younger et al., 2002) (Chapter 7). On the other hand, metal mine drainage remediation should be based on heavy metal removal from the water column and sediment, which require more complicated methods still under development (Nuttall and Younger, 2000, Younger et al., 2002, Johnston and Rolley, 2008).

6 COAL MINE DRAINAGE REMEDIATION: SEDIMENT, WATER AND ECOLOGY OF A RECOVERING STREAM

6.1 Introduction

6.1.1 Mine water remediation

Mine drainage can cause severe damage to recipient stream communities (Chapters 4 and 5). For this reason, a wide range of stakeholders (e.g. mining companies, regulatory agencies) are working to improve efficiency and cost effectiveness of mine water remediation schemes.

There are two main approaches employed to help remediate coal mine drainage: (1) active treatments, which require inputs of continuous energy and/or (bio)chemical reagents, and (2) passive treatments, which although engineered use natural materials to promote chemical and biological processes, manipulating environmental conditions to optimise them (Younger et al., 2002b).

Active techniques have the advantage that the processes involved can be controlled. They are suitable for treatment in working mines or when the mine drainage flow is large and

the land scarce, however they require high maintenance and need regular management, making them more expensive to run than passive techniques (Younger et al., 2002b). The most common active approach for mine remediation is ODAS (Oxidation, Dosing with Alkali and Sedimentation). This technique consists in three phases:

(1) Oxidation, which transforms soluble ions such as Fe^{2+} into oxidised non-soluble forms such as Fe^{3+};

(2) Alkali addition, which raises the pH of the acidic water or buffers pH drop during metal hydrolysis;

(3) Sedimentation, where metal hydroxides are removed from solution.

Passive treatments have the advantage that they are often more cost effective, however they require more land than active techniques and can be less controlled. The most commonly used are engineered wetlands. There are three main types of wetlands, designed to remediate different types of pollutants:

1. Aerobic wetlands are more applicable to ferruginous, net-alkaline waters. They remove iron and manganese, although the latter to a limited extent, via oxidation, hydrolysis and sedimentation (Younger et al., 2002b). However, as shown in equations 6.1-6.3 this hydrolytic process generates protons, thus lowering pH. For this reason, this technique is only applied to net-alkaline waters or to acidic waters previously neutralised. Aerobic wetlands consist of shallow water bodies typically supporting vegetation communities of *Phragmites australis*, *Typha latifolia* and

Juncus effusus (Younger, 2000). Macrophytes appear to assist in water remediation by reducing the flow, providing adsorption surfaces and releasing oxygen into the water (Batty et al., 2008). Additionally, they have proved to be crucial as a polishing step, removing iron when concentrations are too low to be removed by abiotic processes (Batty and Younger, 2002).

$$2FeS_2(s) + 7O_2(aq) + 2H_2O \longrightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \quad (6.1)$$

$$2Fe^{2+} + 1/2O_2 + 2H^+ \longrightarrow 2Fe^{3+} + H_2O \quad (6.2)$$

$$2Fe^{3+} + 6H_2O \longleftrightarrow 2Fe(OH)_3(s) + 6H^+ \quad (6.3)$$

2. In contrast, compost wetlands are suitable for net-acidic waters. They are very similar to aerobic wetlands, but a compost layer is added to provide an organic substrate that supports sulphate reducing microbial communities. In this anoxic substrate, sulphate reduction and calcite dissolution take place, decreasing acidity and removing iron and zinc as sulphides (Younger, 2000). A rise in pH leads to aluminium and manganese precipitation as (hydr)oxide and carbonate respectively (Younger, 2000).

3. Reducing and alkalinity producing systems (RAPS). RAPS are similar to compost wetlands, but have an additional limestone gravel bed, which maximises alkalinity generation (Batty and Younger, 2004). The compost layer holds sulphur reducing bacteria, produces alkalinity and allows insoluble Fe^{3+} to be converted to soluble Fe^{2+} before reaching the limestone bed, avoiding coating of the bed with ochre

which would reduce its reactivity. Thus, RAPS are normally followed by aerobic wetlands to remove dissolved Fe^{2+} from the water. These systems are more efficient in removing trace metals from solution than compost wetlands on their own, however they require the water to flow downwards through the system and not superficially, therefore it is only applicable if there is enough head in the system (Batty and Younger, 2004).

Passive treatments are effective at removing iron from acidic and alkaline waters, but their efficacy with respect to other contaminants is still the subject of on-going research (Younger et al., 2002a, Johnston and Rolley, 2008).

The strategy used to treat coal mine water discharging into Smithy Brook, the stream studied in this chapter, is a combination of active and passive techniques (common in wetlands built by The Coal Authority): the water is initially pumped from Pemberton mine (active), but is then treated via aerobic wetlands (passive) (see section 6.2.1 for more information).

6.1.2 Stream recovery

The impact of a disturbance on a stream and its subsequent recovery not only depends on the chemical and physical properties of the disturbance, but is also a function of the:

a) Physical characteristics and chemical properties of the recipient stream or river. For example, limestone bedrock can provide a natural pH buffer to streams (Cairns et al., 1971).

b) Biological characteristics of the stream. Communities composed of mainly taxa tolerant to mine drainage (such as the Ephemeropteran *Baetis rhodani* and Plecopteran *Amphinemura sp.*) may be more resistant than those where more sensitive taxa (such as Ephemeropteran *Heptagenia dalecarlica*) predominate (Arnekleiv and Storset, 1995).

c) The severity and duration of the stress (Cairns et al., 1971). There are two main types of disturbances depending on their temporal scope: Press disturbances, which are chronic and may involve changes in the watershed and the river channel; and pulse disturbances, which have a limited time scale and do not modify the geomorphology of the stream (Whiles and Wallace, 1992, Milner, 1994).

Ecological recovery of a stream does not always follow chemical or physical recovery of the environment. For example, Bradley et al. (2002) observed that after lime neutralisation of acidic streams, water chemistry improved, but biological recovery did not follow. Lack of ecological recovery after environmental conditions are improved to a favourable condition may be due to several factors:

a) Residual toxicity associated with the disturbance (Gray, 1997). Schultheis et al. (1997) found that organic matter decomposition rates did not recover after copper input into the stream had ceased and water quality improved. This was attributed to residual toxicity of copper incorporated in the biofilm covering the organic matter present in the stream. Similarly, trace metals adsorbed on sediments could pose a threat for benthic community once water chemistry has improved.

b) Habitat and niche loss or modification. This can be due to high sedimentation rates, such as ochre precipitation, common in coal mine drainage impacted streams (Gray, 1997), and may persist after water quality improvement.

c) Availability of undamaged areas to serve as sources for re-colonising organisms (Cairns et al., 1971). Drift or crawling from tributaries and clean headwaters are the main pathway of re-colonisation of streams after perturbations (Herricks and Cairns, 1977, Caines et al., 1985, Skinner and Arnold, 1990, Whiles and Wallace, 1992, Arnekleiv and Storset, 1995, Schultheis et al., 1997, Courtney and Clements, 2002). Re-colonisation can also occur through aerial migration from

downstream and from adjacent streams, in ably dispersing taxa (e.g. Caddis flies) that fly to seek oviposition sites (Blakely et al., 2006).

d) Availability of suitable ovipositing habitat (Blakely et al., 2006). Limnephilidae (caddisfly) have shown site-specific oviposition behaviour, some species preferring protruding stones, others overhanging vegetation (Hoffmann and Resh, 2003).

e) Life history characteristics of the macroinvertebrates inhabiting the stream. Univoltine and semivoltine (one and less generations per year) species may be eradicated from streams with multiple pollution episodes, whereas multivoltine (more than one generation per year) species may be more resilient (Whiles and Wallace, 1992).

f) Remnant species present in the stream after disturbance. These are the organisms left in the stream after the disturbance, and are potentially the initiators of succession. Thus, they can interact with re-colonising species, facilitating or inhibiting their settlement, directly through species interactions or indirectly through ecosystem engineering (Ledger et al., 2006). For example, snails have been seen to play a role in ecosystem engineering: they clear stony surfaces during feeding and facilitate colonisation by simuliids, as availability of surface where simuliids can attach increases (Ledger et al., 2006).

g) Community closure. It is thought that, once a community is formed, addition of new species may be limited (Lundberg et al., 2000). This can become a problem when the organisms left after a disturbance fill niches abandoned by sensitive species and do not allow or limit reinvasion (community closure) (Ledger and Hildrew, 2005). .

The time scales needed for ecological recovery from mine pollution after remediative actions are put in place seem to be highly variable amongst streams, perhaps reflecting the complexity, possible synergies and interactions of the factors discussed above. Some studies report recovery within days or weeks after water quality restoration (Matthaei et al., 1996, Schultheis et al., 1997, Wiseman et al., 2002, Nesbitt, 2006, Dsa et al., 2008), others within 1 to 5 years (Arnekleiv and Storset, 1995, Watanabe et al., 2000) but in some recovery has not occurred after 10 years (Dsa et al., 2008).

In summary, the ecological recovery of a stream depends on many factors not always related to the impact itself, and not always being proportional to stream water chemical improvement. Thus, the recovery pattern may vary in each stream. For this reason, chemical monitoring of stream recovery may not be sufficient, without complementary ecological information.

6.1.3 Is clean water or sediment the key to recovery?

Identifying which specific characteristics of mine pollution have the greatest effect on aquatic communities will improve our ability to optimise and assess remediation strategies and associated recovery. Numerous authors identify contaminated sediments as a threat for benthic organisms (e.g. Bervoets et al., 1997, Bervoets et al., 1998, Kelly, 1999, Battaglia et al., 2005) and as a source of pollution via remobilisation (Kelly, 1999, Dsa et al., 2008). Residual toxicity from biofilm and sediments in clean waters has been suggested to be the reason for inhibition of ecological recovery in some streams (Schultheis et al., 1997, Dsa et al., 2008). In coal mine drainage impacted streams, iron concentration in sediments also appears to reduce shredder abundance (Chapter 5).

However, the relative role of sediment toxicity in mine pollution is equivocal, and some studies identify water as the main stressor on freshwater macroinvertebrates (Courtney and Clements, 2002, DeNicola and Stapleton, 2002, Battaglia et al., 2005). These studies showed that colonisation of contaminated sediments placed in clean streams is fast (up to 5 weeks), and they suggest that once water quality improves, sediments recover (Courtney and Clements, 2002, DeNicola and Stapleton, 2002, Battaglia et al., 2005, Dsa et al., 2008). This appears to be due to ochre flocs being washed away (DeNicola and Stapleton, 2002), although they might accumulate and cause damage further downstream (Dsa et al., 2008). However, the experiments undertaken in these studies consist of placing trays with contaminated sediments in clean streams, and therefore are in a much smaller scale than the impacted streams. Thus, they have a great water-sediment ratio, and

the time needed for sediment recovery in streams (smaller water-sediment ratio) may increase dramatically (Dsa et al., 2008). Therefore, sediment effect on ecological recovery of streams could be important and needs to be further investigated.

6.2 Aims and objectives

This chapter aims to investigate the recovery of a stream severely impacted by coal mine drainage after wetland installation. The main objectives are to:

1. Establish if there is water and sediment quality recovery after the remediation scheme becomes operational;

2. Determine response times and spatial variations for the different environmental variables (water and sediment) related to mine pollution;

3. Investigate if there is ecological recovery and establish a timescale.

6.3 Methodology

6.3.1 Site description

Pemberton mine discharge (Plate 6.1) enters Smithy Brook to the east of Blundell's Wood, near Wigan (Figures 6.1 and 6.2; SD56080 03490). From this point, the brook flows within an engineered channel (Plate 6.2), with very low habitat quality. Approximately 400m downstream from the mine discharge, the brook enters the urbanised area of Goose Green, Wigan (Figure 6.2), and 2km downstream it flows into River Douglas. Ochre stains in Smithy Brook sediments have been observed until the point where it reaches River Douglas (Wilson, 2004).

Smithy Brook runs though several urbanised areas upstream from the study site (Figure6.1). Directly upstream from Pemberton mine discharge there is a semi-natural woodland area, Blundell's Wood, where the reference sites are located (Figure 6.2). This area is currently infested by Japanese Knotweed (*Fallopia japonica*) and Himalayan Balsam (*Impatiens glandulifera*). During the sampling period (from January 2007), a campaign for the elimination of Japanese Knotweed was undertaken at the second and third sampling points upstream from the mine discharge (U2 and U3) restricting access. EA campaign to control Japanese Knotweed invasion in the UK involves application of pesticides. When the area to treat is near or in water courses, formulations of glyphosate or 2,4-D Amine pesticides are used, these are approved to be used near water and thus no

significant negative impact on Smithy brook macroinvertebrates should be expected (EA 2008).

Pemberton coal mine closed down in the mid 1960s (Wilson, 2004) and it is not the only colliery in the area. There are a number of other mine discharges into Smithy Brook known collectively as Summersales mine approximately 1km upstream from Pemberton mine. Summersales mine discharge is on the agenda of The Coal Authority (CA) for remediation, and during the sampling campaign some pumping trials were undertaken by the CA to establish if all mine discharges could be controlled from one single point. The pumping tests were run from the 28th of January 2007 until the 30th of April 2007, and pumped 60-20 l/s of mine water with iron loadings of 110ppm initially and 60ppm at the end of the trial (Carl Banton personal comm.). During these trials, the stream bed upstream from Pemberton mine discharge was stained orange by ochre deposits (Plate 6.3).

Pemberton mine remediation scheme (Figure 6.2) has been designed to eliminate up to 40ppm of iron in the water by precipitating it as ochre. A pumping station located adjacent to Smithy Brook, where the mine water emerges, leads the water into an aeration cascade and then into two settlement ponds where most of the iron (up to 30ppm) is expected to precipitate as ochre (Wilson, 2005). These are followed by three constructed aerobic wetlands which are expected to function as a final polishing step, removing up to 10ppm of iron (Wilson, 2005).

Rainfall during winter 2007 and especially summer 2007t in the study area was higher than the climatic averages reported by the Mett Office (Table 6.1). Even though hydrological variables are not included in the present study, this fact will be taken into consideration for results interpretation.

Table 6.1 Comparison between rainfall averages (1971-2000, Mett Office) and rainfall measurements during the sampling months (2006-2007) in the study area (data from the Mett Office, 2009)

Year	**Month**	2006-2007 (Bradford station)	1971-2000 average (Manchester airport station)
2006	June	11.6	66.8
	July	29.5	59.5
	August	92.7	70.9
	September	82.7	69.9
	October	89.6	86.0
	November	78.6	81.9
	December	133.5	81.9
2007	January	142.4	71.5
	February	103.4	51.8
	March	49.4	64.0
	April	4.2	49.1
	May	68.8	53.8
	June	261.4	66.8
	July	93.3	59.5
	August	34.3	70.9

Plate 6.1 Pemberton mine untreated discharge entering Smithy Brook (Source: Original)

Plate 6.2 Smithy Brook downstream from Pemberton mine discharge flowing in a man-made channel (site D1) (Source: Original)

Plate 6.3 Smithy Brook flowing through Blundell's Wood (upstream from Pemberton mine discharge) with ochre deposits covering the river bed. February 2007, during pumping trials in Summersales mines (Source: Original)

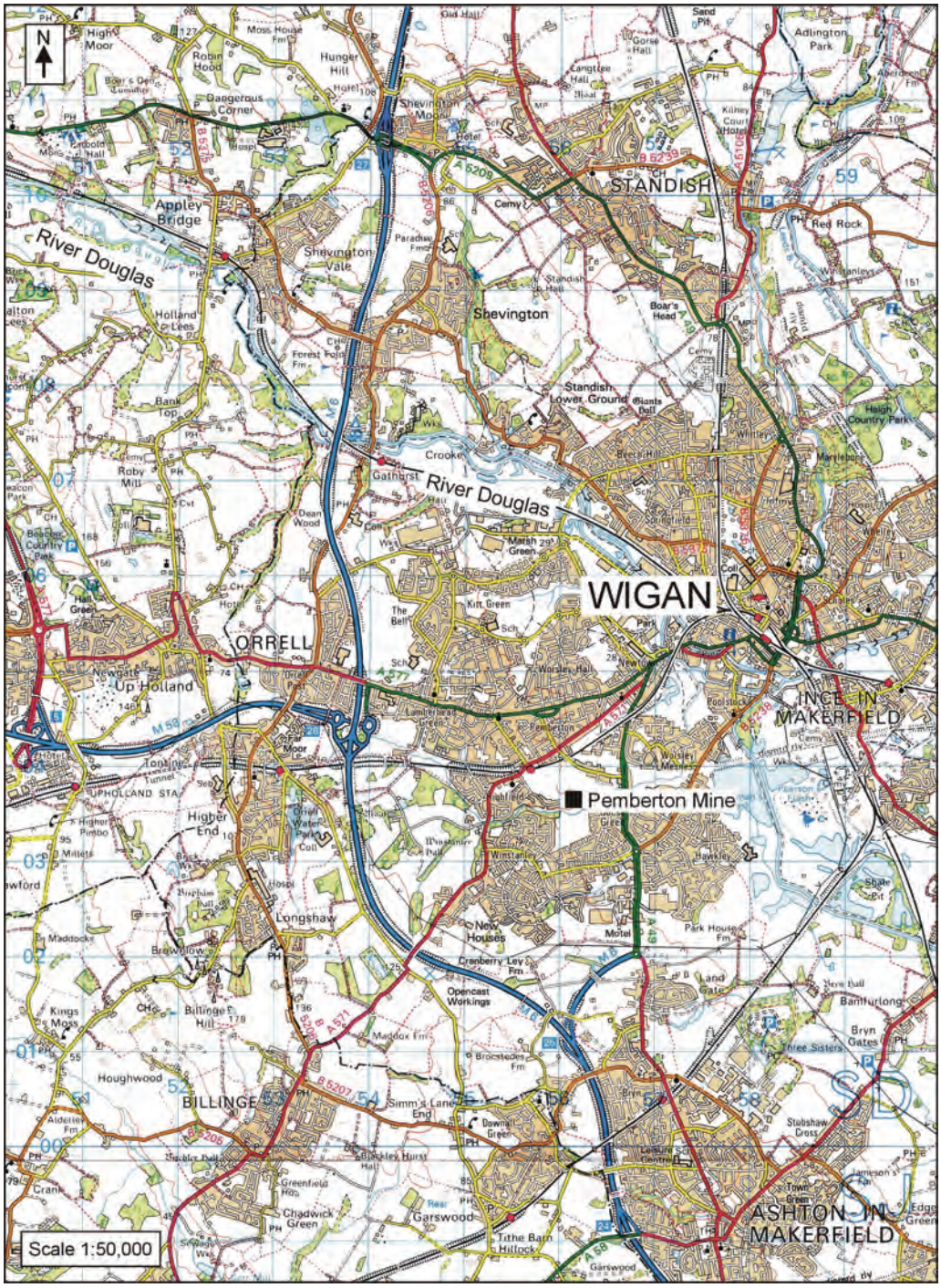


Figure 6.1 Map locating Pemberton mine in Wigan (Manchester)

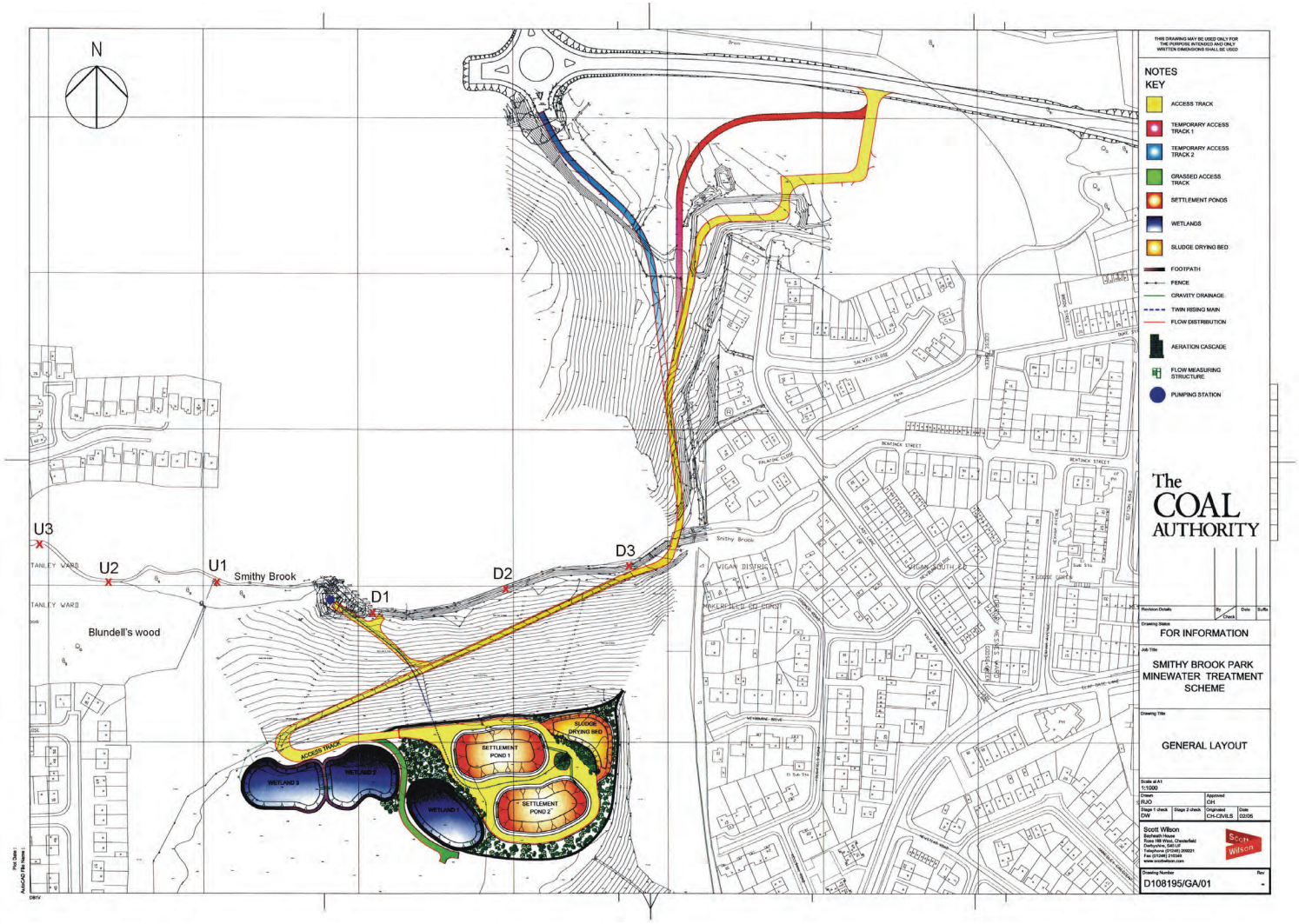

N
U3
U2
U1
D1
D2
D3
Smithy Brook
Blundell's wood
TANLEY WARD
ACCESS TRACK
SETTLEMENT POND 1
SETTLEMENT POND 2
SLUDGE DRYING BED
WETLAND 1
WETLAND 2
WETLAND 3
NOTES
KEY
ACCESS TRACK
TEMPORARY ACCESS TRACK 1
TEMPORARY ACCESS TRACK 2
GRASSED ACCESS TRACK
SETTLEMENT PONDS
WETLANDS
SLUDGE DRYING BED
FOOTPATH
FENCE
GRAVITY DRAINAGE
TWIN RISING MAIN
FLOW DISTRIBUTION
AERATION CASCADE
FLOW MEASURING STRUCTURE
PUMPING STATION
The COAL AUTHORITY
FOR INFORMATION
SMITHY BROOK PARK MINEWATER TREATMENT SCHEME
GENERAL LAYOUT
Scale at A1 1:1000
Drawn RJO
Approved GH
Stage 1 check DW
Stage 2 check
Originated CH-CIVILS
Date 02/05
Scott Wilson
D108195/GA/01
Rev -

Figure 6.2 (Previous page) Map of the study area showing Pemberton mine remediation scheme installations and sampling points in Smithy Brook. U1, U2 and U3 are located upstream from the mine discharge in Blundell's Wood, and D1, D2 and D3 downstream from the mine discharge but upstream from the residential area. Map courtesy of The Coal Authority

6.3.2 Sampling strategy

A BACI (Before/After/Control/Impact) (Underwood, 1996) design was adopted to address the study aims. Three reference sampling points (U1, U2 and U3) were located upstream from Pemberton mine discharge within Blundell's Wood (Figure 6.2). At these sites, one water sample was taken monthly (from June 2006 until August 2007) to analyse major cations and anions, trace metals and suspended sediments (SS), and these were coupled with *in situ* hydrochemical measurements (conductivity (EC), total dissolved solids (TDS), pH, discharge, temperature and dissolved oxygen (DO)) (Section 2.2, Chapter 2). From January and February 2007, access to U3 and U2 respectively was restricted due to works undertaken in the area to eliminate Japanese Knotweed. Hence, only data from U1 are available for the rest of the survey, but these three sampling points do not show significant differences in water hydrochemistry.

Three sampling points were located downstream from Pemberton mine discharge (D1, D2 and D3) in order to identify spatial (longitudinal) differences in water and sediment quality and macroinvertebrate assemblages (Figure 6.2). Due to possible inputs from urbanised areas which may affect water and ecological quality in the stretch of stream

studied, only the 400m before the brook reaches the residential area were sampled (Figure 6.2). These downstream sites were sampled monthly (between June 2006 until August 2007) for water, sediments, macroinvertebrate (four Surber samples per site), and *in situ* hydrochemical measurements (EC, TDS, pH, DO, temperature and discharge) (following methodology in Chapter 2).

Sediments and ecology were not sampled in the upstream sites because the habitat (deep channel with very fine sediment) was completely different to that found downstream from Pemberton mine (shallow man-made channel with coarse sediments) and both habitats were not considered comparable for the present study. Additionally, the main aim of this chapter is to investigate recovery downstream from the mine discharge after remediation is implemented, not upstream downstream differences investigated in previous chapters. Thus, sampling and analysis of sediments and ecology required time and effort that was not considered necessary for the scope of this study. Water chemistry was sampled upstream as a cost-effective control measure. Table 6.2 summarises the comparisons investigated in this study.

Table 6.2 Comparisons analysed in the study of the recovery of Smithy brook before and after coal mine drainage remediation

	Upstream versus downstream	Before versus after remediation
Water chemistry	X	X
Trace metals in Sediment		X
Ecology		X

Sampling of the waters coming out of the mine and those from the wetland once in operation was difficult due to access restrictions. Only in April 2007, it was possible to sample both outputs, the results are displayed in Table 6.3.

Table 6.3 Water chemistry of Pemberton mine drainage as it comes out of the ground (mine output) and once it has gone through the engineered wetland (wetland output). Data from April 2007

	Mine output	Wetland output
Fe (ppb)	21660	13.38
Cr (ppb)	BD	0.25
Mn (ppb)	1474	1121
Ni (ppb)	158.20	11.76
Cu (ppb)	1.76	2.14
Zn (ppb)	8.12	6.87
Cd (ppb)	BD	BD
Pb (ppb)	BD	BD
pH	6.65	7.89
EC (µS)	1794	1722
TDS (ppm)	1333	1272
Na (ppm)	200.90	199.10
Mg (ppm)	58.15	57.25
K (ppm)	16.64	16.43
Ca(ppm)	104.30	101.50
Chloride (ppm)	38.45	35.18
Nitrate (ppm)	0.58	0.02
Sulphate (ppm)	620.45	615.99
Phosphate (ppm)	0.26	0.13

6.3.3 Data analysis

6.3.3.1 Statistical analysis

Paired comparisons with Wilcoxon signed rank test were carried out between water hydrochemistry at the reference site (average of the three upstream sites) and at each downstream site. Data were classified in three periods:

- From June 2006 until December 2006 (prior to remediation): to evaluate the impact of untreated Pemberton mine drainage on Smithy Brook (n=7; Table 6.4).
- From January 2007 until April 2007: during pumping trials (n=4; Table 6.5).
- From May 2007 until August 2007 (after remediation started): to establish if Pemberton wetland is successful in the remediation of the mine waters (n=4; Table 6.6).

Only the first and third periods were considered for the study of water remediation at Pemberton wetland, although, trends in the second and third period were similar.

6.3.3.2 Temporal and spatial variation

Iron, manganese, pH, conductivity, TDS and SS in water were plotted for each month and site (upstream and downstream) to study their spatial and temporal distribution. The effect of coal mine pollution, pumping trials of Summersales mines and the remediation scheme were studied to establish response times for each element and water chemistry recovery at Smithy brook.

Oligochaeta, Chironomidae and *Baetis rhodani* were the main taxa found at the study site. For the ecological study, only chironomids and *B. rhodani* were considered. Chironomidae species richness and abundance, *B. rhodani*, iron and manganese concentrations in sediments were plotted for each month downstream of Pemberton mine discharge. Their recovery and response times were investigated after the treatment scheme became operational.

6.4 Results

6.4.1 Chemistry variation downstream from the mine discharge: before and after treatment

Chemical data obtained from Smithy Brook are presented in Tables 6.4-6.6, separating the three periods described in the methodology: before mine water treatment (Table 6.4), during pumping trials (Table 6.5) and during water remediation (Table 6.6). Paired comparisons (upstream versus downstream) before and after remediation identify those hydrochemical variables modified by the treatment scheme (Tables 6.4 and 6.6). The period during pumping trials (Table 6.5) was excluded from this analysis. From June 2006 until December 2006, before water remediation started (Table 6.4), EC, TDS, SS, sulphate, sodium, calcium, magnesium, potassium, manganese and iron significantly ($p<0.05$) increase downstream from Pemberton mine, whereas DO, pH, chloride and nitrate decrease ($p<0.05$).

From May 2007 to August 2007, when water remediation was taking place (Table 6.6), no significant differences are apparent in pH, DO, trace metals, SS and TDS concentration between upstream and downstream sites. However, EC, sulphate, calcium, magnesium, sodium and potassium increase downstream from the mine discharge, and chloride and nitrate decrease ($p<0.05$).

Table 6.4 Water and sediment chemistry (mean ± SD, n=7) of Smithy Brook prior to mine water treatment (from June until December 2006). U: average of the 3 sampling points upstream from the mine discharge. D1, D2 and D3: three sites downstream from the mine discharge

	U	D1	D2	D3
pH	7.5 ± 0.3	7.0 ± 0.1^{+}	7.1 ± 0.1^{+}	7.0 ± 0.2^{+}
EC (µS/cm)	711 ± 66	1093 ± 205*	1066 ± 201*	1096 ± 173*
DO (%)	99 ± 1	92 ± 11*	100 ± 13	103 ± 5
TDS (ppm)	532 ± 60	777 ± 152*	804 ± 149*	786 ± 121
Fe (ppb)	64 ± 101	4905 ± 4302*	1169 ± 1114*	265 ± 384*
Mn (ppb)	321 ± 86	730 ± 119*	667 ± 118*	615 ± 165*
Fe sediment (mg/kg)		109000 ± 47000	91000 ± 38000	85000 ± 1000
Mn sediment (mg/kg)		2100 ± 1070	2160 ± 870	2060 ± 1260
Cu sediment (mg/kg)		480 ± 550	260 ± 120	140 ± 40
Ni sediment (mg/kg)		1000 ± 750	570 ± 260	300 ± 160
Pb sediment (mg/kg)		100 ± 100	260 ± 160	90 ± 50
Zn sediment (mg/kg)		340 ± 140	380 ± 280	380 ± 60
SS (ppm)	7.5 ± 3.4	15.1 ± 4.8*	16.9 ± 7.5	14.7 ± 6.9*
Na (ppm)	29.8 ± 5.0	104.1 ± 11.8*	87.5 ± 30.3*	83.4 ± 25.2*
Mg (ppm)	24.5 ± 3.7	37.5 ± 3.3*	34.5 ± 7.5*	33.2 ± 6.5*
K (ppm)	6.0 ± 0.5	10.4 ± 0.7*	9.4 ± 2.0*	9.1 ± 1.5*
Ca (ppm)	76.0 ± 13.0	89.1 ± 13.8*	86.8 ± 21.0*	82.7 ± 11.9
Cl^{-} (ppm)	40.9 ± 5.6	38.9 ± 4.1^{+}	38.7 ± 3.8^{+}	39.2 ± 4.4^{+}
NO_3^{-} (ppm)	8.2 ± 4.2	5.5 ± 2.4^{+}	6.5 ± 3.7^{+}	6.6 ± 3.7^{+}
SO_4^{2-} (ppm)	129.3 ± 39.0	228.2 ± 83.0*	205.8 ± 99.0	207.1 ± 98.3
PO_4^{3-} (ppm)	0.03 ± 0.05	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00

*value of the variable recorded downstream from the mine discharge is significantly higher than that recorded upstream according to Wilcoxon sign rank test

$^{+}$value of the variable recorded downstream from the mine discharge is significantly lower than that recorded upstream according to Wilcoxon sign rank test

Table 6.5 Water and sediment chemistry (mean ± SD, n=4) of Smithy Brook during pumping trials further upstream (from January until April 2007). U: average of the 3 sampling points upstream from the mine discharge. D1, D2 and D3: three sites downstream from the mine discharge

	U	D1	D2	D3
pH	7.2 ± 0.4	7.4 ± 0.3	7.2 ± 0.5	7.0 ± 0.6
EC (µS/cm)	94 ± 134	1182 ± 70	1086 ± 166	1151 ± 249
DO (%)	111 ± 27	102 ± 5	100 ± 4	97 ± 15
TDS (ppm)	932 ± 93	1182 ± 50.0	1086 ± 121	1151 ± 175
Fe (ppb)	4856 ± 5747	3614 ± 6984	2837 ± 5105	2599 ± 4783
Mn (ppb)	887 ± 279	1020 ± 247	956 ±222	918 ± 265
Fe sediment (mg/kg)		85000 ± 20000	99000 ± 52000	152000 ± 41000
Mn sediment (mg/kg)		4800 ± 2500	4400 ± 2500	2800 ± 2600
Cu sediment (mg/kg)		80 ± 60	70 ± 60	100 ± 30
Ni sediment (mg/kg)		430 ± 450	220 ± 280	50 ± 80
Pb sediment (mg/kg)		110 ± 210	90 ± 110	450 ± 440
Zn sediment (mg/kg)		400 ± 120	300 ± 190	490 ± 230
SS (ppm)	19.3 ± 1.5	15.1 ± 0.9	17.2 ± 0.0	15.5 ± 0.0
Na (ppm)	33.0 ± 5.4	99.9 ± 17.0	73.6 ± 18.9	71.3 ± 23.3
Mg (ppm)	37.7 ± 8.9	47.1 ± 7.1	44.5 ± 7.0	42.7 ± 11.1
K (ppm)	7.6 ± 1.1	11.4 ± 1.3	10.0 ± 1.4	9.6 ± 2.3
Ca (ppm)	88.9 ± 18.0	96.2 ± 11.9	96.4 ± 9.9	93.0 ± 17.7
Cl^- (ppm)	37.6 ± 2.9	36.3 ± 2.5	36.9 ± 2.8	37.1 ± 3.1
NO_3^- (ppm)	5.2 ± 2.6	3.4 ± 1.7	4.4 ± 2.4	4.6 ± 2.5
SO_4^{2-} (ppm)	276.0 ± 93.3	396.8 ± 69.4	344.2 ± 93.0	343.2 ± 101.5
PO_4^{3-} (ppm)	0.07 ± 0.06	0.08 ± 0.02	0.06 ± 0.08	0.05 ± 0.02

Table 6.6 Water and sediment chemistry (mean ± SD, n=5) of Smithy Brook after remediation of mine waters started (from May until August 2007). U: average of the 3 sampling points upstream from the mine discharge. D1, D2 and D3: three sites downstream from the mine discharge

	U	D1	D2	D3
pH	7.6 ± 0.9	7.7 ± 0.4	7.7 ± 0.5	7.8 ± 0.6
EC (µS/cm)	742 ± 0	1322 ± 250*	1118 ± 189*	1118 ± 193*
DO (%)	101 ± 0	102 ± 6	109 ± 2	108 ± 7
TDS (ppm)	2905 ± 3359	965 ± 187	809 ± 138	491 ± 594
Fe (ppb)	230 ± 126	251 ± 132	332 ± 58	307 ± 235
Mn (ppb)	183 ± 223	473 ± 157	368 ± 169	355 ± 164
Fe sediment (mg/kg)		83000 ± 33000	48000 ± 38000	56000 ± 16000
Mn sediment (mg/kg)		4000 ± 33000	3000 ± 38000	2000 ± 16000
Cu sediment (mg/kg)		150 ± 100	60 ± 60	70 ± 30
Ni sediment (mg/kg)		680 ± 420	320 ± 400	390 ± 370
Pb sediment (mg/kg)		130 ± 120	190 ± 200	240 ± 240
Zn sediment (mg/kg)		420 ± 160	260 ± 240	350 ± 80
SS (ppm)	5.4 ± 1.7	6.6 ± 1.5	5.7 ± 2.3	5.5 ± 2.6
Na (ppm)	31.9 ± 6.2	107.7 ± 40.1*	87.2 ± 32.6*	83.7 ± 31.8*
Mg (ppm)	26.8 ± 7.1	40.0 ± 10.7*	37.2 ± 10.5*	35.9 ± 9.8*
K (ppm)	6.0 ± 0.6	10.0 ± 3.0*	9.1 ± 2.0*	9.5 ± 1.4*
Ca (ppm)	59.2 ± 17.9	65.1 ± 21.0*	72.5 ± 25.5*	63.6 ± 21.7
Cl^- (ppm)	20.6 ± 23.6	13.4 ± 14.5$^+$	17.5 ± 19.2$^+$	17.4 ± 19.1
NO_3^- (ppm)	5.2 ± 1.2	2.1 ± 0.3$^+$	2.6 ± 0.5$^+$	2.6 ± 0.5$^+$
SO_4^{2-} (ppm)	96.9 ± 119.8	279.1 ± 169.4*	264.9 ± 110.6*	263.5 ± 127
PO_4^{3-} (ppm)	0.09 ± 0.05	0.08 ± 0.01	0.11 ± 0.09	0.10 ± 0.05

*value of the variable recorded downstream from the mine discharge is significantly higher than that recorded upstream according to Wilcoxon sign rank test

$^+$value of the variable recorded downstream from the mine discharge is significantly lower than that recorded upstream according to Wilcoxon sign rank test

6.4.2 Temporal and spatial variation of water chemistry: response times

Figure 6.3 depicts iron spatial and temporal variation upstream and downstream from Pemberton mine discharge. Iron concentrations are higher at the downstream sites than upstream. Downstream from Pemberton mine, iron concentration in water appears to be variable until March 2007, with one clear peak (10-14ppm) in February 2007, from this point onwards the concentration is low (<1ppm) and constant. Upstream from Pemberton mine discharge, iron concentration is low and constant (<1ppm) during all the sampling period except between February 2007 and April 2007.

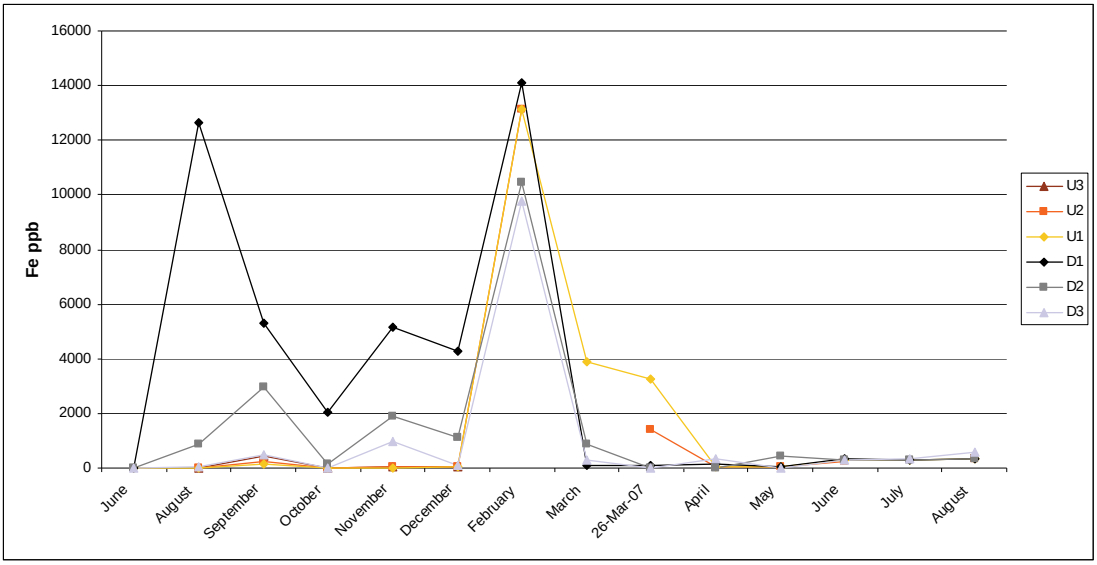


Figure 6.3 Temporal and spatial changes in iron concentration (ppb) in Smithy Brook water at three sampling points upstream (U) and three downstream (D) from Pemberton mine discharge

Figure 6.4 displays spatial and temporal variations of manganese in Smithy Brook water upstream and downstream from Pemberton mine discharge. From June 2006 until December 2006, upstream and downstream manganese concentrations are clearly differentiated, being constantly higher at the downstream sites. From January 2007 onwards, manganese concentrations at the reference point and downstream from the mine discharge converge. Manganese concentrations present a peak in February (12-13 ppm) followed by a steady decline reaching concentrations of 400-500 ppb in August 2007, similar to those in summer 2006 (400-880 ppb).

Figures 6.5-6.8 display pH, EC, TDS and SS temporal and spatial variations in Smithy Brook upstream and downstream from the mine discharge. Similarly to iron and manganese concentrations in water, pH and SS readings upstream and downstream converge from January 2007 onwards. In the case of EC and TDS, readings converge after January 2007, but seem to diverge again from April 2007.

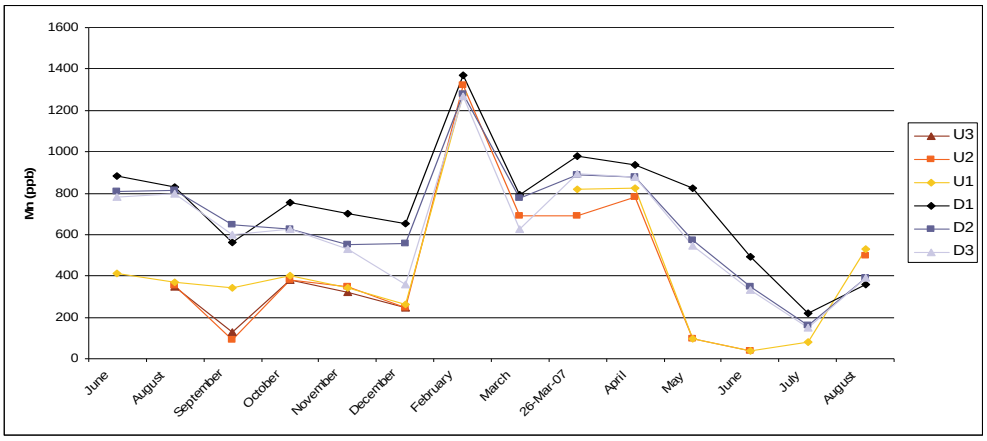


Figure 6.4 Temporal and spatial changes in manganese concentration (ppb) in Smithy Brook water at three sampling points upstream (U) and three downstream (D) from Pemberton mine discharge

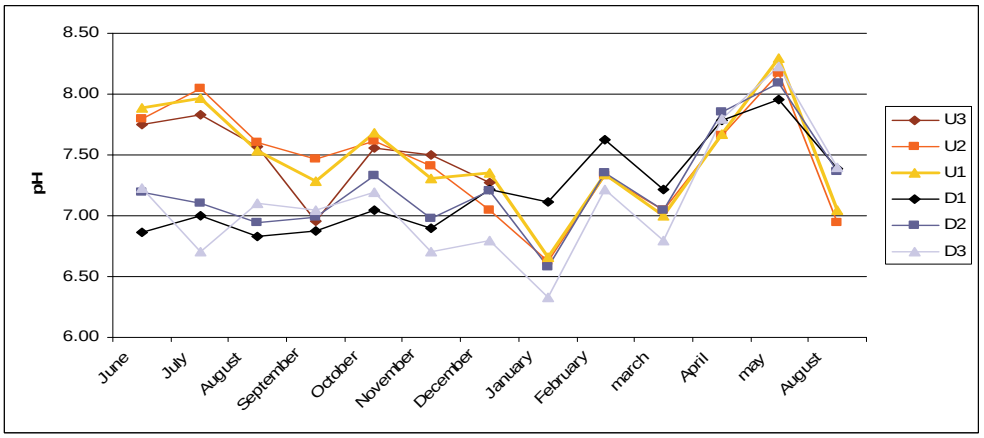


Figure 6.5 Temporal and spatial changes in pH in Smithy Brook water at three sampling points upstream (U) and three downstream (D) from Pemberton mine discharge

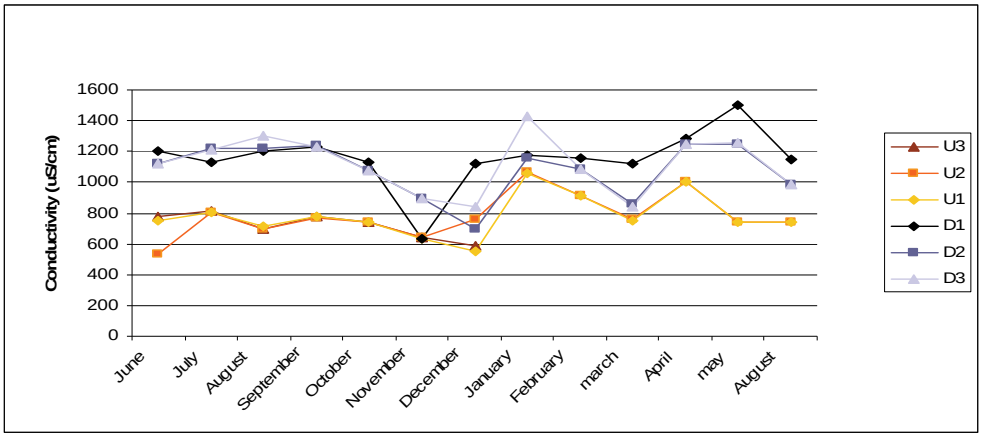


Figure 6.6 Temporal and spatial changes in conductivity (µS) in Smithy Brook water at three sampling points upstream (U) and three downstream (D) from Pemberton mine discharge

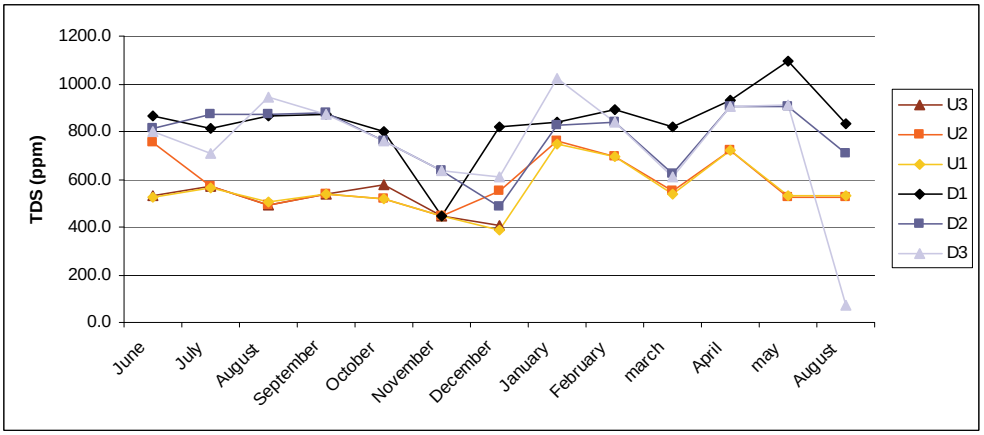


Figure 6.7 Temporal and spatial changes in total dissolved solids concentration (ppm) in Smithy Brook water at three sampling points upstream (U) and three downstream (D) from Pemberton mine discharge

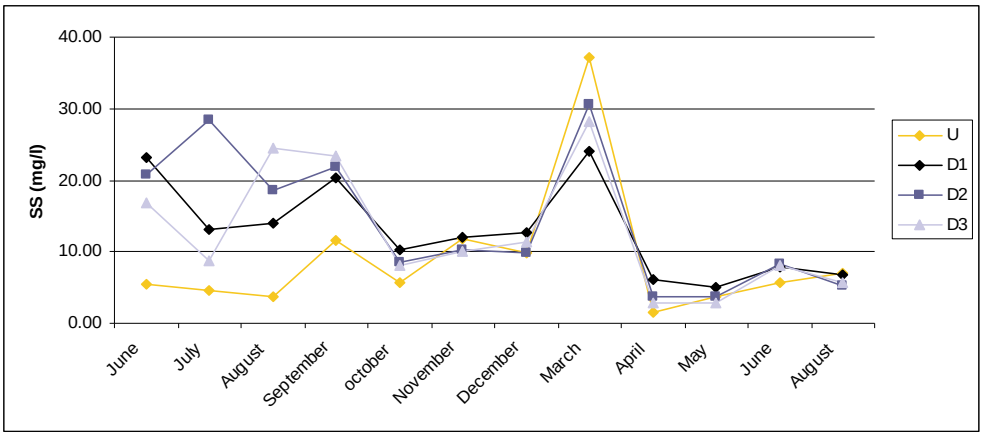


Figure 6.8 Temporal and spatial changes in suspended sediments concentration (ppm) in Smithy Brook water at three sampling points upstream (U) and three downstream (D) from Pemberton mine discharge

6.4.3 Temporal and spatial variation of sediment chemistry: response times

Figure 6.9 depicts the temporal and spatial variation of iron concentration in sediments collected at Smithy Brook downstream from Pemberton mine, this appears to increase from 60000-85000 mg/kg Fe in August 2006, up to 200000 mg/kg Fe between January 2007 and March 2007. A subsequent drop (down to 20600 mg/kg Fe) during April 2007 and May 2007 is followed by a steady increase until reaching values of up to 130000mg/kg Fe in August 2007, when sampling ceased.

Figures 6.10 to 6.13 depict spatial and temporal variations of manganese, nickel, lead and zinc concentrations in sediments collected downstream from Pemberton mine discharge. None of the figures display a clear pattern.

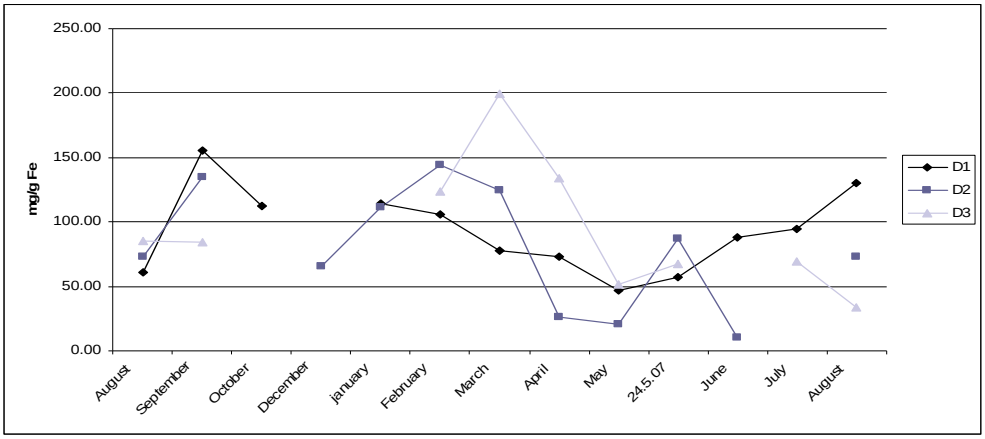


Figure 6.9 Temporal and spatial changes in iron concentration (mg/g) of sediments in Smithy Brook at three sampling points downstream (D) from Pemberton mine discharge

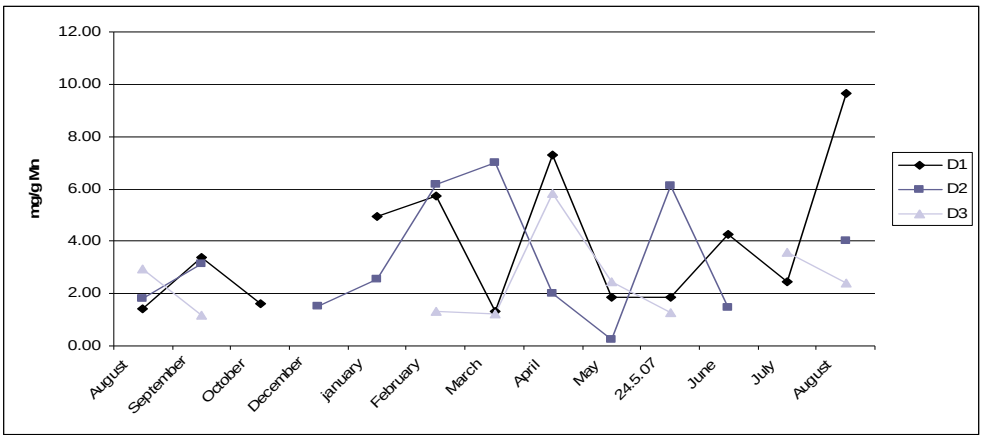


Figure 6.10 Temporal and spatial changes in manganese concentration (mg/g) of sediment in Smithy Brook at three sampling points downstream (D) from Pemberton mine discharge

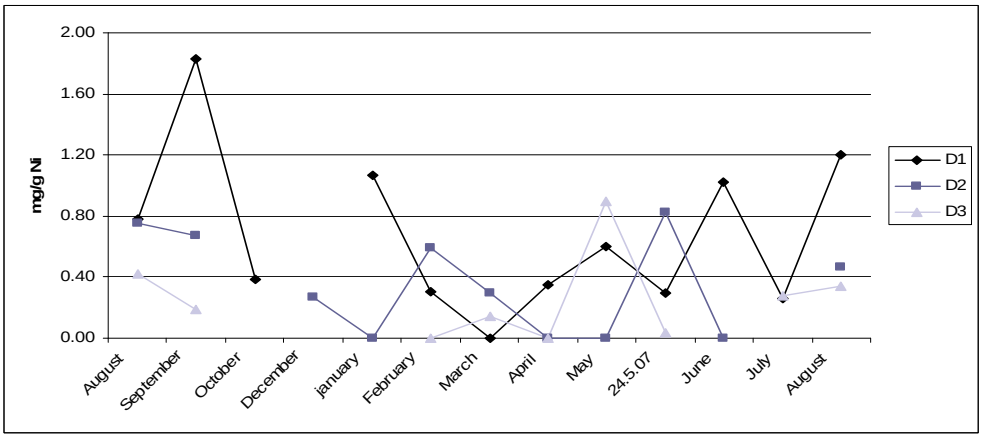


Figure 6.11 Temporal and spatial changes in nickel concentration (mg/g) of sediment in Smithy Brook at three sampling points downstream (D) from Pemberton mine discharge

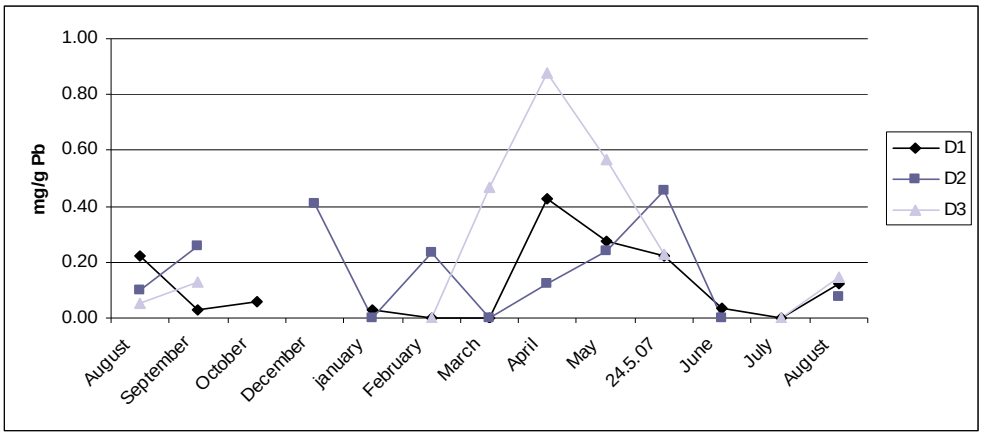


Figure 6.12 Temporal and spatial changes in lead concentration (mg/g) of sediment in Smithy Brook at three sampling points downstream (D) from Pemberton mine discharge

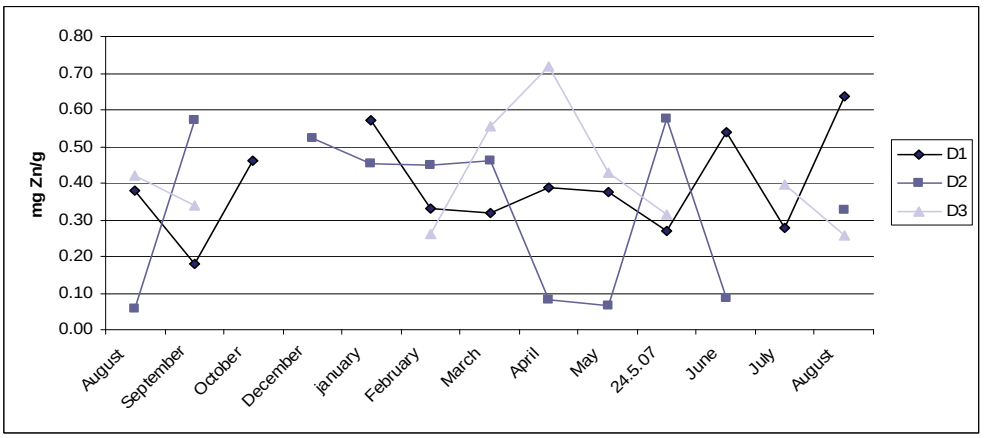


Figure 6.13 Temporal and spatial changes in zinc concentration (mg/g) of sediment in Smithy Brook at three sampling points downstream (D) from Pemberton mine discharge

6.4.4 Temporal and spatial variation of stream ecology: recovery time and distance

Chironomidae and Oligochaeta were the main macroinvertebrate taxon recorded at the study site prior to water remediation. Due to the low diversity of macroinvertebrates, chironomids have been used as an indicator group to study the recovery of the stream. *Baetis rhodani* was also recorded in Surber samples collected in Smithy Brook, especially after June 2007 (Figure 6.16).

Figure 6.14 depicts spatial and temporal variation in Chironomidae species richness at the three sites downstream from Pemberton mine discharge. No consistent differences are found in species richness, nor abundance (Figure 6.15), between the 3 sampling points.

Chironomidae species number decreases during colder months (from 6-10 species in June 2006 down to 1-3 species in January and February 2007). After April 2007 (remediation started) there is a sharp increase of Chironomidae species to approximately 10, where it stabilises until August 2007, when sampling ceased.

Figure 6.15 depicts the spatial and temporal distribution of Chironomidae abundance downstream from Pemberton mine discharge. Chironomidae abundance is very low during the colder months, from September 2006 until April 2007 (<30 individuals) (mine drainage not being treated). A sharp increase in abundance occurs in May 2007 (up to 390 individuals) (treatment started), with a subsequent steady decrease to 55-74 individuals in August 2007. Chironomidae abundance is higher in summer 2006 and 2007 than in the

colder months, however the number of individuals recorded in 2006 never exceeded 100 as in 2007.

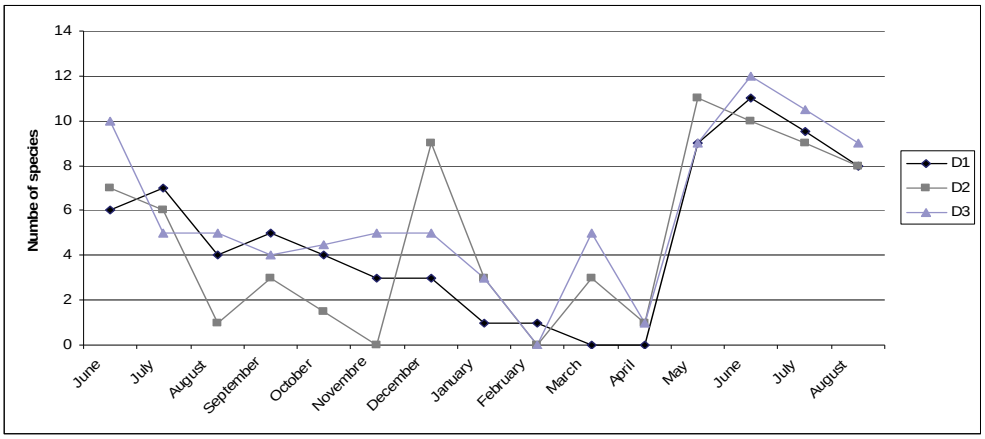


Figure 6.14 Spatial and temporal variation of Chironomidae species richness downstream (D) from Pemberton mine discharge in Smithy Brook from June 2006 until August 2007

Baetis rhodani was not recorded in Smithy Brook for most of autumn and winter (from August 2006 until May 2007) (mine drainage not treated), but, similarly to Chironomidae, increased sharply (up to 634 individuals in D2) in June 2007 (treatment started), a month later than chironomids. At the end of the summer season, when sampling ceased at the study site, *B. rhodani* numbers seem to decrease, but are still generally higher (between 89 and 475 individuals) than records from the previous summer (151 individuals in D2 in June 2006).

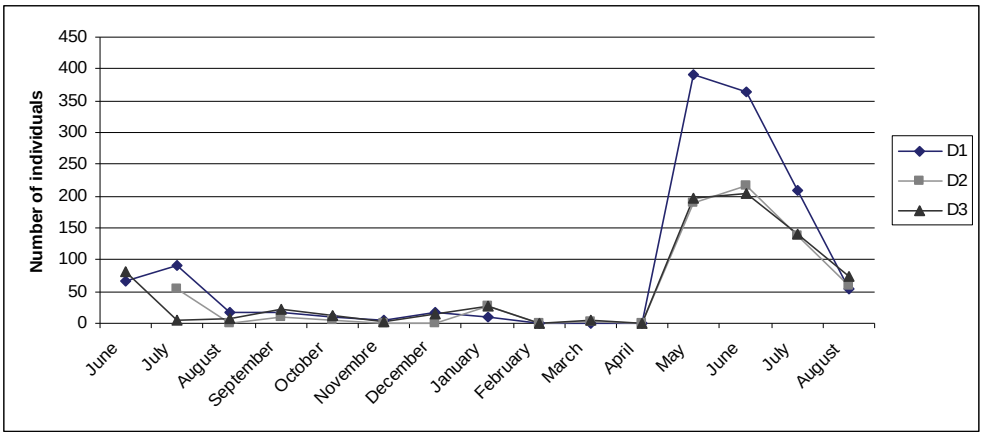


Figure 6.15 Spatial and temporal variation of Chironomidae abundance downstream (D) from Pemberton mine discharge in Smithy Brook from June 2006 until August 2007

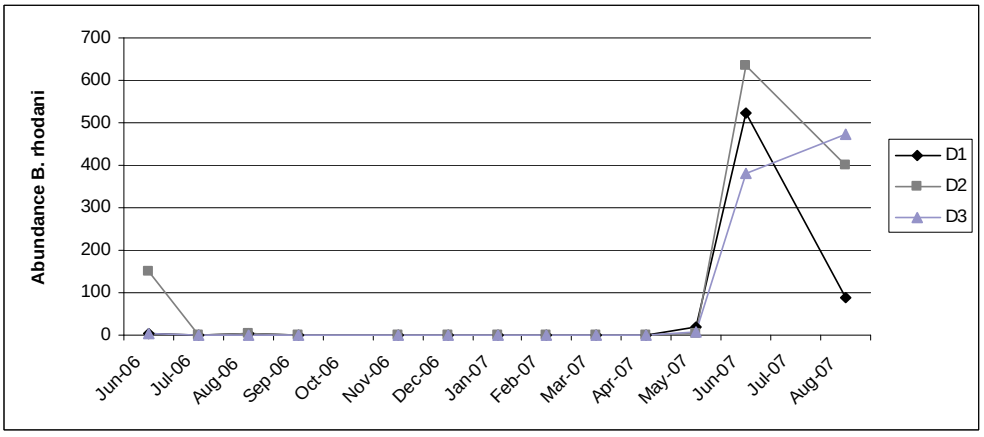


Figure 6.16 Temporal and spatial changes in *Baetis rhodani* total abundance in Smithy Brook at three sampling points downstream (D) from Pemberton mine discharge

6.5 Discussion

6.5.1 Chemistry variation downstream from Pemberton mine discharge: before and after treatment

Coal mine drainage discharge increases sulphate, TDS, SS, iron, manganese, sodium, calcium, magnesium and potassium concentrations, and decreases pH and DO in receiving streams from South West Wales (Chapter 3). Smithy Brook presented similar hydrochemical characteristics when Pemberton mine drainage discharged into the stream without treatment (data from June 2006 to December 2006), thus coal mine pollution appears to be the main factor controlling Smithy Brook water chemistry.

Iron concentration in Smithy Brook water before remediation started (January 2007) increases downstream from the mine discharge. During this period, iron concentration is highly variable, suggesting that there might be some effect from variations in river flow, mine discharge volume or chemical composition. Consistent data on the mine water discharge during the study period were not available due to access restrictions. However, the CA monitored Pemberton mine discharge in 2004, prior to the design of the wetland, recording concentrations between 20-30ppm with monthly variations of up to 10ppm (Wilson, 2004). This suggests that iron concentration in the mine discharge may not be constant during the study period. Manganese also increases downstream from Pemberton mine discharge during this period, however its concentration is more constant in time.

Similarly, the CA data show a rather constant manganese concentration of 1.6-1.8ppm during the monitoring in 2004 (Wilson, 2004).

Compared to upstream reference sites, DO is significantly reduced only at D1, suggesting a quick recovery when stream water is in contact with the atmosphere. Additionally, DO readings were always close to saturation values (>92%), hence no ecological response was expected.

Sulphate appears to follow a similar pattern to DO, presenting some recovery further downstream from the mine discharge. Sulphate is known to co-precipitate with iron oxides forming a metastable solid, $Fe(OH)_{2.35}(SO_4)_{0.325}$ (Langmuir, 1997). Hence, extensive ochre precipitation (associated with SS in stream water, Chapter 3) observed downstream from Pemberton mine discharge might have removed sulphate from the water column, explaining sulphate decrease in concentration along the length of the stream. This contradicts Rikard et al. (1990) suggestion that sulphate is a good mine pollution indicator due to its persistence in the environment. However, at Smithy Brook, this persistence in sulphate seems to only occur after treatment starts, when sulphate concentrations are still high at all downstream sites (>263.5 ± 127ppm), but SS (between 2.8 and 8.2ppm from May to August 2007) and iron (<1ppm) decrease (see below). During this period, sulphate may not have co-precipitated with iron oxides and thus persisted in the environment.

Chloride and nitrate decrease downstream from the mine discharge during the whole sampling campaign, suggesting a dilution of these anions by the mine water. Smithy Brook flows through urbanised areas before reaching the study area, although not within the stretch sampled for this study (upstream and downstream from Pemberton mine discharge). Thus, remnants of urban pollution may be expected in the stream water, although no new input should occur within the study area. High concentrations of chloride and nitrate recorded upstream from Pemberton mine discharge may represent this remnant urban pollution. Chloride is one of the main components in road salts (Olofsson and Lundmark, 2009), whereas nitrate origins are various, from waste water treatment plants, industrial sewage (Flintrop et al., 1996) and septic tanks (Wernick et al., 1998), to dry deposition of atmospheric nitrogen oxides (Howarth et al., 1996). Nitrate and chloride concentrations significantly decrease downstream from the mine discharge. Nitrate concentration in Pemberton mine drainage in April 2007 was very low (0.02 ppm), suggesting that this may dilute the higher concentration in Smithy Brook water (1.77ppm upstream in April 2007). On the other hand, chloride concentration recorded in mine water in April 2007 (38.45ppm) is only slightly lower than that recorded in Smithy Brook upstream from the mine discharge in the same month (39.95ppm), although this difference may increase in other months.

Data after May 2007 have been used to test remediation success at Pemberton mine. These data show no difference in trace metals, DO, acidity, SS and TDS between downstream and reference sites, demonstrating the efficiency of the engineered wetland installed in treating the main aspects of coal mine pollution.

During this period, iron concentration (from about 5ppm originally to less than 1ppm), SS (from around 15ppm to less than 6.6ppm) and pH (from around 7 to 7.8) are successfully remediated. Manganese concentration in Smithy Brook water also appears to be reduced (from about 700ppb down to 4ppb), however the response pattern is slightly more complicated and will be discussed in the next section. Both iron and manganese concentrations are below the Environmental Quality Standards (EQS) derived from the Water Framework Directive (Table 1.1, Chapter 1) of 1ppm for iron and 17.5ppb for manganese.

On the other hand, EC, sulphate, sodium, magnesium, potassium and calcium still increase downstream from Pemberton wetland discharge, suggesting that these components of the mine water are not being treated within the scheme. Sulphate is above the EQS of 87.5ppm both upstream (average of 96.9ppm) and downstream (average of 263.5-279.1ppm) of Pemberton mine discharge, probably as a result of the input from Summersales mines located about 1km upstream from the study site.

Previous work undertaken in coal mine impacted streams as part of the present project (Chapters 4 and 5) suggests that EC, sulphate and major cations are not responsible for the main negative effects of coal mine drainage pollution on freshwater invertebrates. SS,

pH and iron in sediments seem to be the most harmful components. However, the strong negative impact of pH, SS and iron may mask a possible negative effect of water electrolyte status that may become apparent once these are treated. Negative effects of sulphate and conductivity have been reported in other studies (e.g. Goetsch 1996, Buzby 2007) and will be further discussed in section 6.5.4.

6.5.2 Temporal and spatial variation of water chemistry: response times

Data from January to August 2007 (including pumping trials in Summersales mine and operation of Pemberton remediation scheme) have been studied to gain more knowledge about response times of several pollutants in Smithy Brook, and to understand and predict hydrochemical recovery of streams impacted by coal mine drainage after remediation implementation.

Iron appears to have very quick response times. Peaks in iron concentration both upstream (up to 13 ppm) and downstream (up to 14 ppm) from Pemberton mine discharge appear at the same month pumping trials at Summersales mine started. Iron concentration in the water pumped out of Summersales mine was not constant thorough the whole period, from an initial 110ppm of iron it dropped to 60ppm (Carl Banton personal comm.) as the galleries were emptying and water had less residence time within the mine, therefore dissolving less pyrite. This is likely to be the reason why iron concentration at the study stretch of Smithy Brook decreases during the last two months of the pumping trials (March and April 2007).

SS also seem to have quick response time. SS temporal pattern appear to mimic iron concentration in waters, probably because most SS are formed by iron precipitating out of the water column as ochre flocs (Chapter 3 and Equations 6.1-6.3).

Manganese response time appears to be longer than in the case of iron, since concentration in Smithy Brook water decreases more slowly after pumping from Summersales mine ceased (April 2007). This delay in response can be due to persistence of manganese in solution at pH below 8 (Lee et al., 2002), since pH above this value was rarely recorded at Smithy Brook during the sampling campaign; or re-mobilisation of manganese adsorbed to sediment due to favourable conditions when water concentration decreases and pH is below 8, as described in other studies (Kelly, 1999, Dsa et al., 2008). Additionally, interactions between manganese and dissolved iron in the stream water may obscure the response patterns: ferrous iron has been seen to dissolve manganese oxides (Villinski et al., 2001) and prevent their formation (Gouzinis et al., 1998), as discussed in Chapter 3.

Conductivity, acidity and TDS do not present clear response patterns to wetland remediation. Their readings appear to converge when pumping trials started and, even though TDS and pH seem to be successfully improved by the wetland, response times for the three components are not obvious from the available data. This could be due to other factors influencing stream water pH and electrolyte status in the whole catchment area (upstream and downstream from the mine discharge), such as rainfall which has naturally

a pH of 5.64 (Moss, 1997), or urban runoff loaded with nitrate, chloride and other salts entering the stream further upstream from the study stretch.

6.5.3 Temporal and spatial variation of sediment chemistry: recovery time and distance

Iron concentration in sediments appears to respond to iron concentration in water, but with some spatial variation. There is an iron concentration peak in D2 and D3, probably corresponding to iron input in Smithy Brook from Summersales mine pumping trials recorded in the stream water. This peak appears in February 2007 in D2 (145600mg/kg), whereas in D3 (200000mg/kg) it appears in March 2007. In D1, this peak could occur in January 2007 (114000mg/kg), but as there are no data available for December 2006 this can not be confirmed.

When pumping trials ceased, low iron concentrations were recorded in the stream sediments (down to 20600mg/kg Fe). However, after May 2007, iron concentration in sediments presents a steady increase to values similar to summer 2006 (up to 130000mg/kg Fe), suggesting that there is still an input of iron in the stream. This input could be sediments further upstream releasing iron into the water which may precipitate downstream and/or re-mobilisation of upstream sediments, as observed in other studies (Kelly, 1999, Dsa et al., 2008), or passive discharge from Summersales mine as water

rebounds and floods the galleries again, continuing with dissolution of pyrite and other minerals.

Even though manganese was detected in water and significantly increased downstream from Pemberton mine discharge and due to Summersales mine pumping trials (see above), this response pattern is not reflected in the sediment. This might be due to manganese limited transfer from water column to sediments, possibly due to its high solubility in aqueous environments with pH < 8 (Lee et al., 2002), and its interactions with ferrous iron (Fe^{2+}), as Fe^{2+} seems to prevent manganese oxide (MnO_2) formation (Gouzinis et al., 1998) and even dissolve MnO_2 (Villinski et al., 2001) (Chapter 3).

Nickel, lead and zinc were not detected in the stream water, hence a trend within sediments was not expected. Their presence in the sediment is probably due to background pollution. Sediments sampled from other coal mine drainage impacted streams in South West Wales as part of this project (Chapter 3) also presented no significant increase of trace metals other than iron downstream from the mine discharge.

The study suggests that during a period of 6 months following water remediation, iron concentration in sediments from Smithy Brook did not decrease. Similarly, Dsa et al. (2008) did not observe any recovery in trace metals contained in stream sediments after 5 weeks of exposure to clean water. However, other studies suggest that once water has been successfully treated, sediments also improve their quality (Courtney and Clements, 2002, DeNicola and Stapleton, 2002, Battaglia et al., 2005). DeNicola et al. (2002) suggest that sulphates adsorbed onto ochre are lost when the sediment is exposed to clean

water, this destabilises the iron precipitate which may be washed away. If sulphate desorption is needed for sediment recovery, the fact that Pemberton wetland does not eliminate sulphate and concentrations in the stream water were still high (>263.5ppm) during remediation, could prevent sediment recovery in Smithy Brook.

Additionally, previous studies investigated stream recovery by placing trays of polluted sediment in clean streams. Under these conditions, the ratio of water-sediment is very high, and this might greatly speed recovery (Dsa et al., 2008). However, the time needed for full stream sediment recovery might be several orders of magnitude higher than in these *in situ* experiments due to a much lower water-sediment ratio (Dsa et al., 2008). Thus, the time scale of the present study (6 months after water remediation) may not be long enough to observe sediment recovery.

6.5.4 Temporal and spatial variation of stream ecology: recovery time and distance

Lotic environments polluted by coal mine drainage typically hold communities formed by Oligochaeta and Diptera, mainly Chironomidae (Gray, 1997), and these are the main two taxon encountered at the study stretch from Smithy Brook before the remediation scheme became operational.

As discussed in the previous sections, Pemberton remediation scheme appears to remediate water successfully, in terms of trace metals, DO, pH and SS. However, the electrolyte status of the water is not modified and sediments do not seem to recover

within the time scale of the present study. Hence, it needs to be investigated if water quality improvement in terms of trace metals, pH and ochre precipitation is enough for ecological recovery of the stream.

Chironomidae present less species richness during colder months than during summer 2006 and 2007 probably due to seasonal differences, as better conditions, in terms of food and water temperature, are found during summer (Jeyasingham and Ling, 2000). The summer during which the remediation scheme was operational (2007) seems to be slightly richer in species (up to 12 species) than the summer prior to water treatment (2006) (up to 10 species), this could be due to yearly differences or water quality improvement.

Chironomidae abundance presents a dramatic increase in the summer when water was being treated (2007) (up to 390 individuals in May 2007 in D1) in comparison to the summer prior to remediation (2006) (<100 individuals), suggesting that water quality improvements lead to better conditions for chironomids. Abundance data suggest that Chironomidae have a recovery time of 3 months from water quality improvement, however as recovery coincides with beginning of summer, life cycles could determine recovery time. In summer, there are more flying adults and thus more chances of colonisation via oviposition (Milner, 1994), additionally, more favourable conditions (e.g. food and temperature) (Jeyasingham and Ling, 2000) may facilitate stream recovery.

Baetis rhodani abundance presents a similar pattern than Chironomidae, increasing sharply during the summer after remediation became operational (July 2007) (from 8-21 individuals in May to 379-634 in June 2007). However, it seems that *B. rhodani* needs one month more than Chironomidae to respond to water quality improvement, with a response time of 4 months. However, similarly to chironomids, baetids recovery time could be determined by life cycles. Several studies have found that *B. rhodani* is one of the first organisms to re-colonise streams when disturbance has ceased (Skinner and Arnold, 1990, Arnekleiv and Storset, 1995, Matthaei et al., 1996). Even though Ephemeroptera are considered to be very sensitive to mine drainage pollution (Arnekleiv and Storset, 1995, Hickey and Clements, 1998, Richardson and Kiffney, 2000, Hickey and Golding, 2002, Van Damme et al., 2008), the genus *Baetis* is thought to be more tolerant (Arnekleiv and Storset, 1995). Additionally, *Baetis* is a common taxa in drift, and motile organisms are likely to be the first ones to re-colonise disturbed patches (Skinner and Arnold, 1990).

Pemberton mine water remediation scheme does not seem to improve the electrolyte status of the mine water, as sulphate concentrations (263-279ppm), conductivity (1118-1322µS/cm) and TDS (491-965ppm) are still high after remediation started (section 6.4.1). Other studies of coal mine drainage remediation have shown that the output waters are high in sulphate and conductivity (Buzby, 2007). Hence, when investigating ecological recovery of streams after mine drainage remediation it is important to take these components into account. Several studies have investigated the effect of TDS and EC on freshwater macroinvertebrates reporting toxic response at conductivities of

100mS/m (within the range of those recorded during this study) (Goetsch et al., 1996) or TDS concentrations of 1000ppm (slightly higher than the average recorded at D1 of 965ppm) (Buzby, 2007). However, these effects highly depend on the ionic composition of the stream water (Chapman, 2000). For example, Goetsch et al. (1996) observed higher toxicity by sulphate than by chloride at similar conductivities, and Kennedy et al. (2005) provided evidence that TDS toxicity is mainly due to sodium and sulphate concentrations in the water column. TDS effect on macroinvertebrates seems to be due to dehydration of gills and internal tissue, alterations of osmotic regulations or disruption of essential enzymatic activity by a specific ion, such as sodium, chloride or sulphate (Goestch et al., 1996, Kennedy, 2003). Therefore, failure in remediation of the electrolyte status of mine waters may prevent ecological recovery of streams even though trace metals and acidity have been eliminated, hence this aspect of coal mine drainage should be taken into consideration in water remediation

The lack of re-colonisation by other taxon than *Baetis* and Chironomidae new species and the delay in their increase in abundance can be due to a number of factors: (a) residual toxicity in the stream sediment (Gray, 1997), as this has not been proven to be remediated during the study period. (b) Negative impact from high sulphate and major cations concentration in water. (c) Lack of undamaged areas serving as sources for re-colonising organisms (Cairns et al., 1971). As it has been mentioned before, coal mine drainage pollution occurs also upstream from the study site, hence drift may be severely reduced. Additionally, since the stream catchment is in a highly urbanised area, there might not exist appropriate nearby water bodies to provide adults for re-colonisation via oviposition.

(d) Lack of suitable ovipositing habitats (Blakely et al., 2006). (e) High ochre precipitation may have eliminated habitats or niches existing in the stream (Gray, 1997), excluding organisms sensitive to fine sediment. (f) Remnant taxa such as Chironomidae and Oligochaeta and coloniser *Baetis rhodani* may prevent reinvasion of other species, following the community closure theory (see Lundberg et al., 2000, Ledger et al., 2006). (g) Habitat at the study stretch from Smithy Brook is poor (man-made channel, Plate 6.2) and this could prevent other taxa from inhabiting the stream, independently of chemical quality. (h) Flooding suffered in the area during the study period (Table 5.1) could also modify macroinvertebrate re-colonisation patterns. (i) The study time scale (six months after remediation starts) may not be long enough to observe ecological recovery.

In summary, when water quality improves, in terms of trace metals, SS, pH and DO, Chironomidae appears to be favoured, increasing in abundance and possibly species richness in 3 months, and *Baetis rhodani* re-colonises the stream in 4 months. However, conditions in Smithy Brook after Pemberton remediation scheme is in operation do not seem to be favourable for other taxa to colonise the stream, at least not during the time scale of this study.

6.6 Conclusion

This study demonstrates that Pemberton engineered wetland is successful in treating coal mine waters in terms of iron, manganese, SS, TDS, DO and acidity. Iron, SS and DO appear to have very quick response times (within the same month of water input changes), whereas manganese, TDS and pH seem to require more time to respond to water treatment. On the other hand, the remediation scheme seems to fail in improving the electrolyte status of the water.

Contrary to what has been suggested in the literature (Courtney and Clements, 2002, DeNicola and Stapleton, 2002, Battaglia et al., 2005), sediment quality does not seem to improve once the stream water was cleaned. Sediments may need more time than water to recover from coal mine pollution once the source is removed, or it may be necessary to actively assist in their remediation. Iron concentration in sediment has proven to be harmful for shredders in streams impacted by coal mine drainage (Chapter 5), hence sediment quality is an important factor to monitor with stream recovery.

The benthic community in Smithy Brook appears to have a poor recovery after water remediation starts. After 3 months of water improvement, Chironomidae seems to increase in abundance, followed by re-colonisation of *Baetis rhodani* a month later. However, there seems to be no re-colonisation by other organisms. The possible reasons for lack of full ecological recovery are numerous, including ecological and physical characteristics of the stream, sediment toxicity, high rainfall during 2007 and water electrolyte status, as discussed above. However, this study suggests that treating mine

water in engineered wetlands may not be enough to gain ecological recovery of a stream within a time scale of 6 months. These findings contradict many studies (Courtney and Clements, 2002, DeNicola and Stapleton, 2002, Battaglia et al., 2005) suggesting that water treatment leads to sediment quality improvement and to ecological recovery, demonstrating how streams present different and unique conditions which will determine the fate of the communities inhabiting in them.

7 CWM RHEIDOL CASE STUDY: WATER, SEDIMENT AND ECOLOGY OF A STREAM IMPACTED BY METAL MINE DRAINAGE

7.1 Introduction

The Environment Agency (EA) Wales, in conjunction with the Welsh Assembly Government and The Coal Authority, is currently managing an EU funded project to remediate Cwm Rheidol mine discharge and improve the ecological status of Afon Rheidol (Edwards and Potter, 2007). This chapter comprises a case study funded by the EA to provide preliminary information on the ecological quality in the river as it flows by Cwm Rheidol mine. The study aims to provide important information on the state of macroinvertebrate communities in the stream prior to remediation, highlighting those aspects that need to be tackled for a successful ecological recovery with the aid of knowledge gained in previous chapters. In order to achieve this aim, macroinvertebrate communities, water and sediment were sampled upstream and downstream from Cwm Rheidol mine discharge, focusing on detecting spatial differences and establishing the effect of spoil heaps on Afon Rheidol.

7.1.1 Mine waste pollution

During mining activity, a considerable amount of waste material is produced, which is generally of two types: waste rock and tailings. Waste rock generally comprises coarse grains (1-50mm) which are often tipped dry, forming spoil heaps (Plate 7.1). Tailings are often finer grained (<1mm) and generally deposited from flowing water (Younger et al., 2002). Both types of waste contain the worked mineral and hence are potential sources of pollution.

The most dramatic mine waste pollution events are normally caused by tailings dam failures, such as the collapse of the dam holding the Aznalcollar tailings pond in Doñana National Park (South Spain) in April 1998, which released 6 million m^3 of mud and acidic water heavily loaded with trace metals into Agrio-Guadiamar river channel and floodplain (Grimalt et al., 1999). However, pollution generated by spoil heaps is more common and widespread (Younger et al., 2002) and will be discussed below.

The chemical processes involved in mineral weathering and dissolution of trace metals in spoil heaps are comparable to those that occur in mine galleries discussed in Chapter 3. The main reactions that can take place in spoil heaps are summarised in equations 7.1-7.4 (see Chapter 3 for further details).

Pyrite is the main mineral in coal mines, but it can also be found with other minerals such as sphalerite in metal mines. During rainfall events, pyrite (FeS_2) found in mine waste rock becomes in contact with highly oxygenated water. Under these conditions, redox reactions transform

sulphur and iron into soluble sulphate and ferrous iron (Equation 7.1), which is then oxidised to ferric iron (Equation 7.2). This precipitates, upon hydrolysis, as iron oxyhydroxide or ochre ($Fe(OH)_3$) (Equation 7.3). Ochre typically covers the river bed polluted by the run off, however it can also precipitate within spoil heaps, staining them orange (Plate 7.1).

$$2FeS_2(s) + 7O_2(aq) + 2H_2O \longrightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \qquad (7.1)$$

$$2Fe^{2+} + 1/2O_2 + 2H^+ \longrightarrow 2Fe^{3+} + H_2O \qquad (7.2)$$

$$2Fe^{3+} + 6H_2O \longleftrightarrow 2Fe(OH)_3(s) + 6H^+ \qquad (7.3)$$

Similar reactions can occur to sulphide minerals other than pyrite, such as sphalerite (ZnS). They do not necessarily produce acidity, but metal ions and SO_4^{2-} are released into the environment. Equation 7.4 displays the example of sphalerite weathering.

$$ZnS + 2O_2(aq) \longrightarrow Zn^{2+} + SO_4^{2-} \qquad (7.4)$$

As waste material in spoil heaps is in greater contact with the atmosphere than the minerals underground, they have the potential to generate more severe pollution than flooded mine galleries (Gandy and Younger, 2008). For example, Fuge et al. (1991) analysed mine water coming out of two adits and runoff from spoil heaps in Cwm Rheidol mine (Mid Wales), concluding that runoff (pH 2.6-2.7, 577-978ppm Zn, 1471-3590ppb Cd, 1200-9350ppb Cu, 1950-2430ppb Ni and 955-1870ppb Pb) contained greater trace metal concentrations and acidity than underground water (pH 2.8-3.7, 9.8-72ppm Zn, 29-112ppb Cd, 30-75ppb Cu, 119-760ppb Ni and

17-1286ppb Pb). Hence, when spoil heaps are present, their impact on stream geochemistry and ecology should be taken into account, as they can cause impacts which can be as severe or greater than those produced by underground mine water.

7.1.2 The riffle-pool sequence: implications for mine pollution

Riffle-pool sequences are common morphological units in gravel and mixed bedded channels of intermediate slope (Emery et al., 2003). However, their method of formation, their specific physical characteristics and their persistence as stable morphologies in alluvial rivers is not fully understood. For the purpose of this chapter, pools are defined as deep sections of a stream, with fine material and slow velocities and, riffles as shallow reaches, with coarser material and higher velocities (Richards, 1982). This definition is generally true during low flow periods, when pools are depositional areas from material eroded in riffles. However, in some streams, during high flow, conditions are thought to change following the flow reversal hypothesis (Richards, 1982). During flow reversal, hydraulic stress in pools exceeds that in riffles, becoming erosional environments, whereas sediments deposit in riffles. This process can have an effect on the ecology of the stream (see below).

Water velocity is not the only factor conditioning benthic fauna in riffle and pools, sediment characteristics and disturbance frequency can also be very important, affecting ecological processes such as:

a) Organism dispersal or drift. Organisms voluntarily use the flow to locate more favourable environments; but high flow velocities can sweep organisms away in a similar manner to sediment erosion and transport (Hart and Finelli, 1999).

b) Food resources. Some organisms need the flow to provide food. For example, filter-feeder ingestion rate of particulate organic matter is limited at low flow velocities, although high velocities may impair their feeding structure (Hart and Finelli, 1999).

c) Respiration. As an adaptation to flowing waters, many lotic macroinvertebrates have lost the ability to ventilate and rely on the current to renew the oxygen supply to their body surface (Hynes, 1970).

d) Habitat selection. Different macroinvertebrate species seem to prefer substrate with different characteristics. For example, filter-feeders tend to inhabit coarser substrates, whereas shredders appear to be common in finer substrates (Hart and Finelli, 1999).

e) Species interactions. Flow velocities may control food supply (see section b) or habitat (see section d), and hence affect competitive interactions. Additionally, flow may determine prey-predator encounter rates or successful capture (Hart and Finelli, 1999).

f) Disturbances. In streams where flow reversal occurs (see above), macroinvertebrates and loose sediments are swept away from pools during high flow. When slower velocities are restored in the pools, fine sediment is deposited between the coarser material, not leaving suitable habitat and refuge for invertebrates that prefer coarse substrate. However, fine

sediments may not be sufficiently abundant or stable for those taxa typically inhabiting fine sediments either (Brown and Brussock, 1991).

As a result, benthos choice of habitat may be a balance between energy costs of maintaining position in a turbulent environment and benefits of sections rich in food and oxygen (Merigoux and Doledec, 2004), with a strong influence of substrate characteristics and disturbance frequency (Brown and Brussock, 1991). This often results in richer and more abundant benthic communities inhabiting riffles (see Brown and Brussock, 1991, Boyero and Bosch, 2004, Costa and Melo, 2008), where conditions (e.g. food, oxygen, substrate stability) tend to be more favourable.

Other studies have compared some ecological processes in riffles and pools, and reached similar conclusions of more abundant and diverse macroinvertebrate communities in riffles. Even though more favourable conditions in riffles could explain this pattern, the authors also identify factors specific to the processes studied which may also be important. For example, Kobayashi et al. (2005) found that leaf packs in riffles sustained more abundant and diverse communities than in pools. They attributed it to the fact that in pools litter deposits due to gravity, whereas in riffles leaves get entangled in the stream bed; therefore, during periods of high flow, pool organic matter is more likely to be swept away, whereas riffle litter may be more stable. Fenoglio et al. (2005) found that salmon carcasses deposited in riffles sustained richer and more abundant communities than those in pools. They suggested that the carcass and fungi growing on it offered refuge to invertebrates while they filter organic particles from the flow.

Therefore, mesohabitats (e.g. pools and riffles) in streams can be very important in determining benthic species composition, and they may complicate detection of the effect of environmental stressors on macroinvertebrates. Nevertheless, few projects include habitat characteristics in the study of pollution. Brabec et al. (2004) found that organic pollution reduced the differences between riffle and pool communities, and although riffles appeared to still hold higher diversity, this might suggest that they are more affected by the disturbance than pools. Buss et al. (2004) found that at the river scale, substrate type (namely pools and riffles) was more important than water quality, environmental degradation and season, in structuring benthic communities. However, water quality was a good predictor for macroinvertebrate assemblages at smaller scales (e.g. between riffles). Similarly, Lorion and Kennedy (2009) observed higher diversity in riffle communities than in pools when studying deforestation effects, but both habitat types responded similarly to environmental degradation. Hence, relative response of macroinvertebrate communities inhabiting pools and riffles appears to be equivocal and needs further investigation.

The accumulation of fine sediments in pools can affect the ecology of the stream directly, for example through habitat selection, but can also determine geochemical properties of the sediments. Sediment sorption capacity is not only controlled by hydrochemical characteristics of the stream (e.g. pH, suspended sediment) (Chapter 3), but also particle size. Smaller sediment particles, which have higher surface-volume ratio, tend to have higher sorption capacities. Clay particles also have a charged surface and therefore a high capacity for cation adsorption (Ladd et al., 1998). It is expected, therefore, that pool sediments will have higher trace metal concentrations than riffle sediments. This has been confirmed by Ladd et al. (1998), where a

sediment trace metal concentration gradient (from higher to lower concentrations) was established as follows: eddy drop zones and attached bars > pools and detached bars > riffles and glides. It is sensible to hypothesise that the effect of metal mine drainage on benthic organisms will differ between riffle and pool environments, due to different properties of the sediment.

7.1.3 Aims and objectives

The objectives of the chapter are to:

a) Determine if Cwm Rheidol mine is negatively impacting on Afon Rheidol water, sediment, and ecological quality;

b) Determine if there is spatial recovery as the river flows downstream from the mine discharge;

c) Investigate differences in water chemistry, trace metal concentration in sediments and ecology between riffles and pools.

7.2 Methodology

7.2.1 Site description

Cwm Rheidol is an abandoned metal mine located in Mid Wales, approximately 16km from Aberystwyth, adjacent to the Afon Rheidol, in the same area as the other metal mines studied in this project (Figure 2.1, Chapter 2). There are records of ore extraction in Cwm Rheidol dating to 1705 (Bick, 1975), however, mining activity in the area goes back to at least Roman times (Bick, 1974). Production in the mine ceased in 1914, although there was some intermittent activity until the mid-20th century (Bick, 1975).

The main ore worked at Cwm Rheidol was sphalerite (ZnS), but the worked material also contained high quantities of iron pyrites (FeS_2) (Bick, 1975), causing the characteristic orange stains in spoil heaps (Plate 7.1) and in the water draining out of the mine (Plate 7.2). Cwm Rheidol mine discharge is thought to contribute to nearly half the loading of trace metals (e.g. zinc and lead) received by the Afon Rheidol (Edwards and Potter, 2007).

In the early 1960s, a limestone filter bed was constructed to treat the main mine discharge, increasing its pH and precipitating metals out of solution. The treatment scheme was initially effective, however in October 1969, a wooden plug damming one of the adits from the mine was accidentally removed, and its drainage blocked the filter with ochre, which has not been operational since then (Fuge et al., 1991) (Plate 7.3).

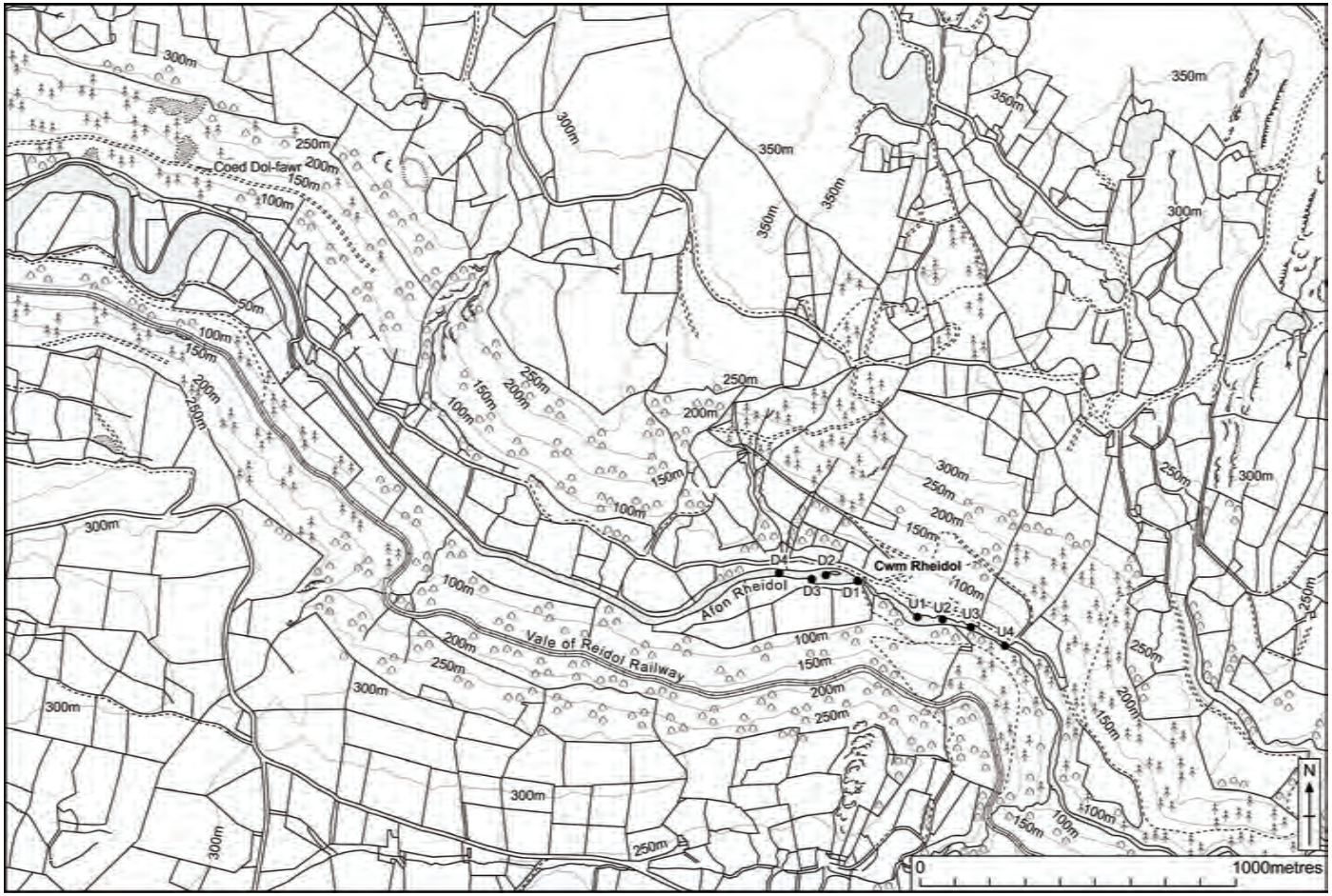


Figure 7.1 Map of the study area showing sampling points upstream (U) and downstream (D) from Cwm Rheidol mine discharge

Plate 7.1 Cwm Rheidol spoil heaps stained with orange, probably due to iron oxides (U2 sampling point) (Source: original)

Plate 7.2 Cwm Rheidol underground mine drainage before it reaches the disused limestone filter bed and flows into Afon Rheidol (Source: original)

Plate 7.3 A disused limestone filter bed constructed in the 1960s to treat Cwm Rheidol mine discharge (Source: original)

7.2.2 Sampling strategy

Four sampling points were established upstream (U) and downstream (D) from Cwm Rheidol mine in order to identify spatial differences (Figure 7.1). Half of these sampling points (U2, U3, D2 and D3) were riffles, whereas the other half (U1, U4, D1 and D4) were pools. In each sampling point the same methodology used in other chapters was followed: sampling of water, sediment and macroinvertebrates, seasonally during one year (Chapter 2).

Major ions in water (chloride, nitrate, sulphate, phosphate, calcium, potassium, magnesium and sodium), trace metals in sediment and water (zinc, lead, iron, manganese, copper, cadmium, nickel and chromium), pH, conductivity, total dissolved solids (TDS), suspended sediments (SS), temperature, dissolved oxygen (DO) and discharge were measured at each sampling point (Chapter 2, Section 2.2). Four Surber samples were collected per sampling point, and macroinvertebrates were identified to species level for Ephemeroptera, Plecoptera, Trichoptera and Chironomidae (Chapter 2, Section 2.4).

7.2.3 Data analysis

Spatial distribution of water chemistry, trace metal concentration in sediment, and benthos abundance and diversity was investigated with the aid of figures where values of each variable in each sampling site and each season were plotted.

Differences in water and sediment chemistry, macroinvertebrate abundance and species diversity between pools and riffles upstream and downstream from the mine drainage discharge were investigated using One-way ANOVA.

7.3 Results

7.3.1 Water chemistry

Tables 7.1-7.3 summarise chemical data of water and sediment collected in Afon Rheidol seasonally between summer 2006 and spring 2007, which is discussed in this chapter.

Zinc, iron and manganese are the main trace metals found in Afon Rheidol water, and the spatial distribution of their concentration at the study stretch is depicted in Figures 7.2-7.4. Other metals analysed (cadmium, copper, nickel, lead and chromium) were not detected in the water samples.

Zinc concentration in water (Figure 7.2) is only above detection limits in winter and spring 2007. In spring 2007 it increases downstream of the mine discharge, however this pattern is not found in winter. Iron concentration in the water appears to be highly variable (Figure 7.3; Table 7.1). In autumn 2006 and winter 2007 iron levels increase downstream from the mine discharge, but this pattern is not found in the other seasons. Manganese was only detected in the samples from Afon Rheidol in winter 2007 and, during this season, no downstream upstream differences can be seen (Figure 7.4).

Sulphate concentration (Figure 7.5) appears to increase at the downstream sites during summer 2006 and spring 2007, but not in the other seasons. Other major ion concentrations (Table 7.2), conductivity and TDS (Table 7.3) appear to be rather constant spatially and do not present any significant trend downstream from the mine discharge.

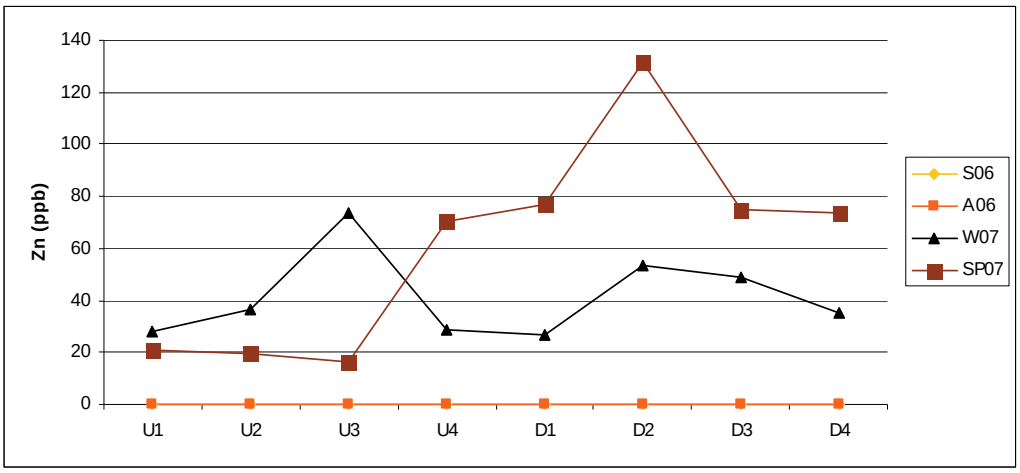


Figure 7.2 Spatial changes in zinc concentration (ppb) in Afon Rheidol water upstream (U) and downstream (D) from Cwm Rheidol mine discharge in summer 2006 (S06), autumn 2006 (A06), winter 2007 (W07) and spring 2007 (SP07)

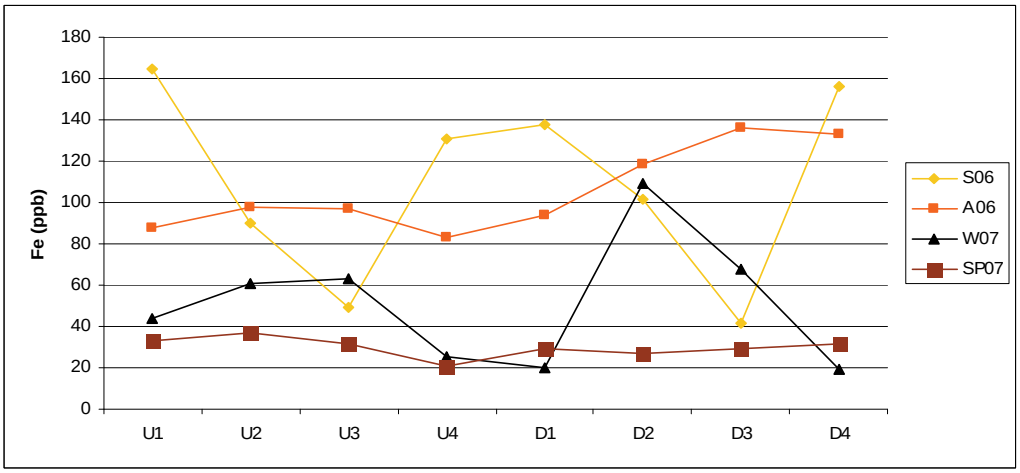


Figure 7.3 Spatial changes in iron concentration (ppb) in Afon Rheidol water upstream (U) and downstream (D) from Cwm Rheidol mine discharge in summer 2006 (S06), autumn 2006 (A06), winter 2007 (W07) and spring 2007 (SP07)

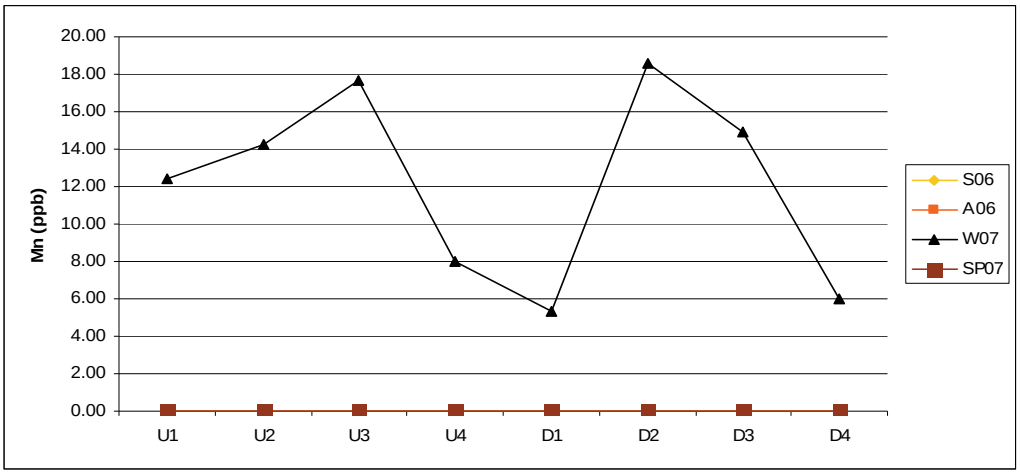


Figure 7.4 Spatial changes in manganese concentration (ppb) in Afon Rheidol water upstream (U) and downstream (D) from Cwm Rheidol mine discharge in summer 2006 (S06), autumn 2006 (A06), winter 2007 (W07) and spring 2007 (SP07)

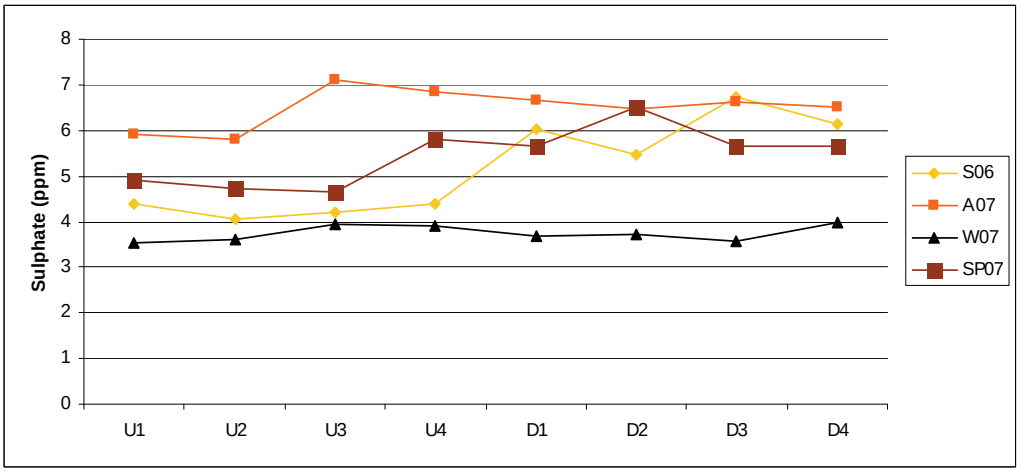


Figure 7.5 Spatial changes in sulphate concentration (ppm) in Afon Rheidol water upstream (U) and downstream (D) from Cwm Rheidol mine discharge in summer 2006 (S06), autumn 2006 (A06), winter 2007 (W07) and spring 2007 (SP07)

Table 7.1 Trace metal annual mean concentrations (± SD, n=4) in water (ppb) and sediments (mg/kg) from Afon Rheidol collected upstream (U) and downstream (D) from the mine discharge

	Water (ppb)			Sediment (mg/kg)			
	Mn	Fe	Zn	Fe	Mn	Pb	Zn
U1	3.1 ± 6.2	82.4 ± 59.9	12.2 ± 14.4	78800 ± 60700	23700 ± 39100	1900 ± 1500	1800 ± 700
U2	3.6 ± 7.1	71.4 ± 27.9	13.8 ± 17.4	94900 ± 26400	13300 ± 9900	1600 ± 2300	2700 ± 2300
U3	4.4 ± 8.8	60.1 ± 27.5	22.6 ± 35.0	68100 ± 44300	21100 ± 14000	400 ± 800	12100 ± 14300
U4	2.0 ± 4.0	65.1 ± 52.0	24.8 ± 33.3	50000 ± 23400	1600 ± 1800	700 ± 600	5000 ± 6100
D1	1.3 ± 2.7	70.3 ± 55.6	25.9 ± 36.2	53300 ± 31500	9000 ± 15100	300 ± 300	3400 ± 4200
D2	4.7 ± 9.3	89.1 ± 42.1	46.1 ± 62.1	70900 ± 8200	15600 ± 18100	1500 ± 800	1800 ± 1100
D3	3.7 ± 7.5	68.6 ± 47.6	30.9 ± 37.2	43000 ± 48700	4900 ± 3300	900 ± 1200	2100 ± 1000
D4	1.5 ± 3.0	85.0 ± 69.4	27.1 ± 35.0	42400 ± 29300	4700 ± 1700	400 ± 100	2400 ± 2400

Table 7.2 Major ion annual means (± SD, n=4) in water (ppm) from Afon Rheidol collected upstream (U) and downstream (D) from the mine discharge

	Na (ppm)	Mg (ppm)	K (ppm)	Ca (ppm)	Chloride (ppm)	Nitrate (ppm)	Sulphate (ppm)	Phosphate (ppm)
U1	4.80 ± 0.41	0.99 ± 0.09	0.08 ± 0.15	2.77 ± 0.92	8.77 ± 2.04	2.44 ± 1.58	4.69 ± 1.00	0.22 ± 0.16
U2	4.74 ± 1.20	0.97 ± 0.25	0.09 ± 0.11	2.26 ± 0.66	8.74 ± 2.12	2.44 ± 1.57	4.56 ± 0.96	0.21 ± 0.15
U3	5.38 ± 0.88	1.19 ± 0.17	0.15 ± 0.13	2.63 ± 0.63	8.74 ± 2.00	2.39 ± 1.52	4.97 ± 1.45	0.18 ± 0.14
U4	4.22 ± 0.82	0.84 ± 0.25	0.10 ± 0.10	1.96 ± 0.99	8.78 ± 2.21	2.16 ± 1.80	5.24 ± 1.33	0.21 ± 0.15
D1	4.43 ± 0.96	0.91 ± 0.36	0.11 ± 0.15	2.54 ± 1.36	8.73 ± 1.87	2.40 ± 1.57	5.50 ± 1.28	0.18 ± 0.17
D2	4.63 ± 0.37	0.98 ± 0.07	0.09 ± 0.12	2.53 ± 0.66	9.19 ± 1.95	2.41 ± 1.46	5.53 ± 1.31	0.18 ± 0.15
D3	4.77 ± 0.82	1.02 ± 0.13	0.09 ± 0.10	2.37 ± 0.57	8.43 ± 1.85	2.36 ± 1.46	5.64 ± 1.47	0.19 ± 0.17
D4	4.25 ± 1.02	0.86 ± 0.86	0.09 ± 0.15	2.18 ± 0.93	8.83 ± 2.18	2.36 ± 1.48	5.57 ± 1.12	0.18 ± 0.16

Table 7.3 Hydrochemistry *in situ* measurements and suspended sediment (SS) concentration (ppm) annual mean (± SD, n=4) in water from Afon Rheidol measured upstream (U) and downstream (D) from the mine discharge

	Discharge (l/s)	pH	Conductivity (µS)	DO (%)	Temperature (˚C)	TDS (ppm)	SS (ppm)
U1	197 ± 115	7.7 ± 0.8	57.0 ± 8.1	101 ±5	10 ± 3	39 ± 6	1.44 ± 2.14
U2	361 ± 271	7.7 ± 0.6	56.5 ± 7.9	105 ± 5	10 ± 4	39 ± 6	1.09 ± 1.25
U3	462 ± 403	7.8 ± 1.0	57.4 ± 9.0	109 ± 8	10 ± 4	39 ± 6	1.53 ± 0.88
U4	542 ± 299	7.7 ± 1.0	58.1 ± 8.3	110 ± 10	11 ± 4	39 ± 6	1.07 ± 0.62
D1	222 ± 181	7.6 ± 1.0	57.7 ± 6.7	109 ± 11	11 ± 5	40 ± 5	0.30 ± 0.39
D2	232 ± 114	7.5 ± 0.8	57.5 ± 6.6	109 ± 10	12 ± 5	39 ± 5	0.84 ± 0.65
D3	436 ± 208	7.4 ± 0.9	57.5 ± 6.7	112 ± 15	12 ± 4	39 ± 5	1.59 ± 1.37
D4	341 ± 174	7.4 ± 1.0	64.7 ± 0.2	104 ± 5	10 ± 3	45 ± 1	1.72 ± 1.77

pH appears to decrease steadily along the whole study stretch during the sampling period, from 7.2-8.9 at U1 to 6.5-8.5 at D4 (Figure 7.6).

Similarly to iron concentrations in water, suspended solids (SS) appear to be highly variable (Table 7.3, Figure 7.7) and present no clear pattern along Afon Rheidol study stretch.

Figure 7.8 does not suggest any differences in stream discharge between upstream and downstream, but Afon Rheidol appears to carry less water in summer 2006 and spring 2007 than during autumn 2006 and winter 2007.

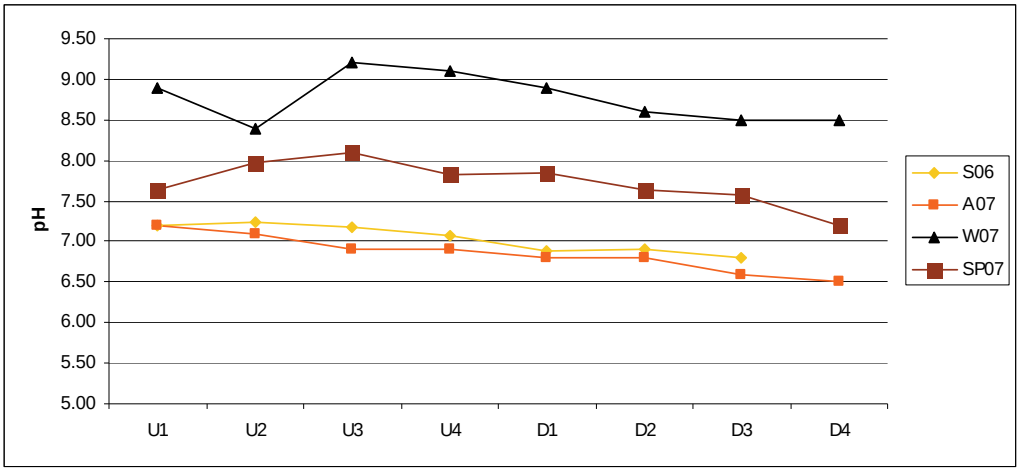


Figure 7.6 Spatial changes in pH in Afon Rheidol water upstream (U) and downstream (D) from Cwm Rheidol mine discharge in summer 2006 (S06), autumn 2006 (A06), winter 2007 (W07) and spring 2007 (SP07)

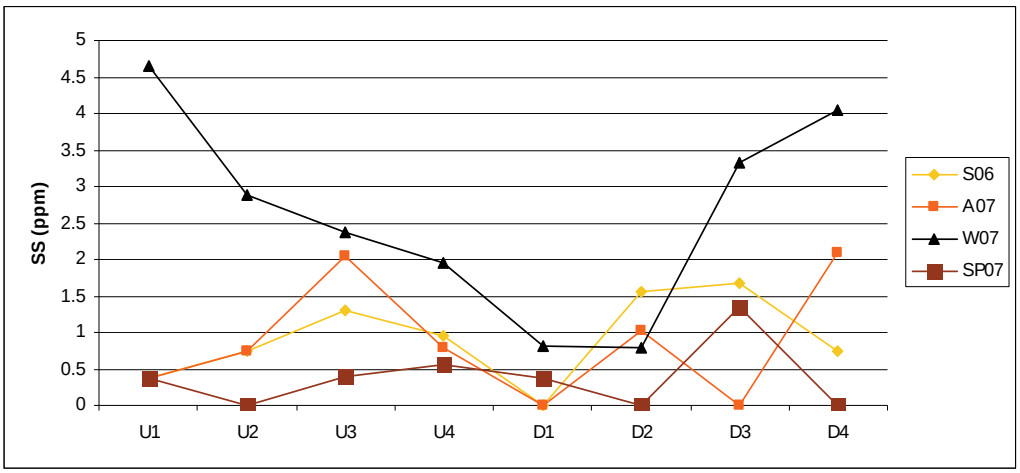


Figure 7.7 Spatial changes in suspended sediments concentration (ppm) in Afon Rheidol water upstream (U) and downstream (D) from Cwm Rheidol mine discharge in summer 2006 (S06), autumn 2006 (A06), winter 2007 (W07) and spring 2007 (SP07)

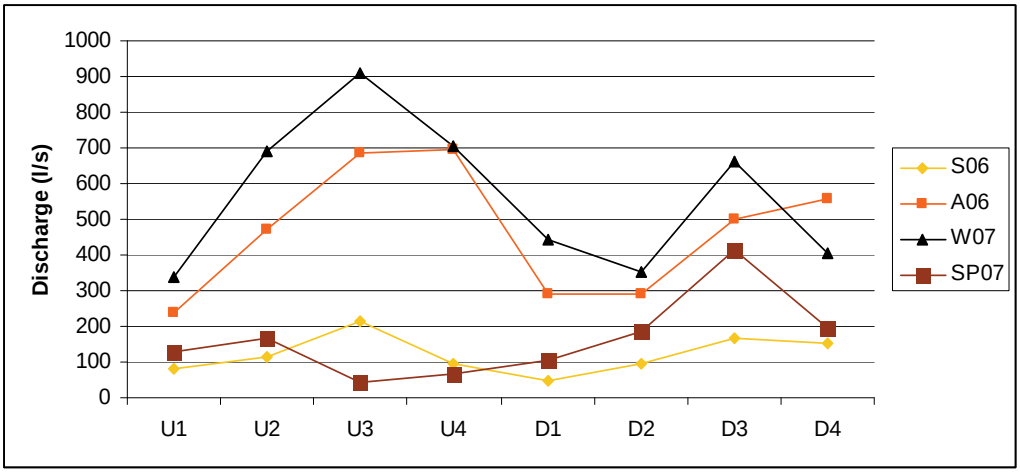


Figure 7.8 Spatial changes in discharge (l/s) in Afon Rheidol water upstream (U) and downstream (D) from Cwm Rheidol mine discharge in summer 2006 (S06), autumn 2006 (A06), winter 2007 (W07) and spring 2007 (SP07)

7.3.2 Trace metals in sediment

Figures 7.9 to 7.12 display annual average concentrations (±1SE, n=8) of trace metals in sediments from pools and riffles upstream and downstream from Cwm Rheidol mine discharge. No significant differences have been detected by One-way ANOVA ($p>0.05$) in any of the trace metal concentrations. However, this seems higher in riffle and pools upstream from the mine dischargethan in their homologue habitats downstream, specially in the case of zinc, iron and manganese. However, the data in each habitat type is too variable (big error bars) for these results to be conclusive.

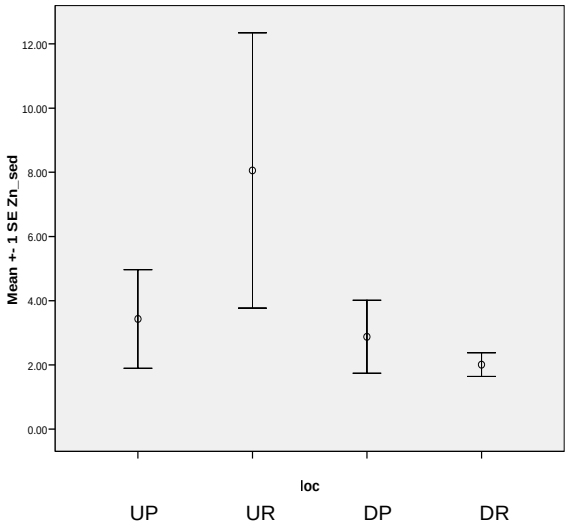


Figure 7.9 Spatial changes in the mean (±1SE, n=8) zinc concentration in sediments (mg/g) upstream (U) and downstream (D) of Cwm Rheidol mine discharge in pools (P) and riffles (R)

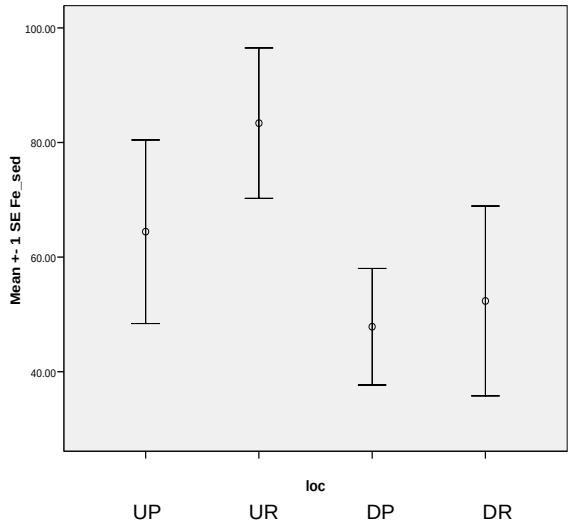


Figure 7.10 Spatial changes in the mean (±1 SE, n=8) iron concentration in sediments (mg/g) upstream (U) and downstream (D) of Cwm Rheidol mine discharge in pools (P) and riffles (R)

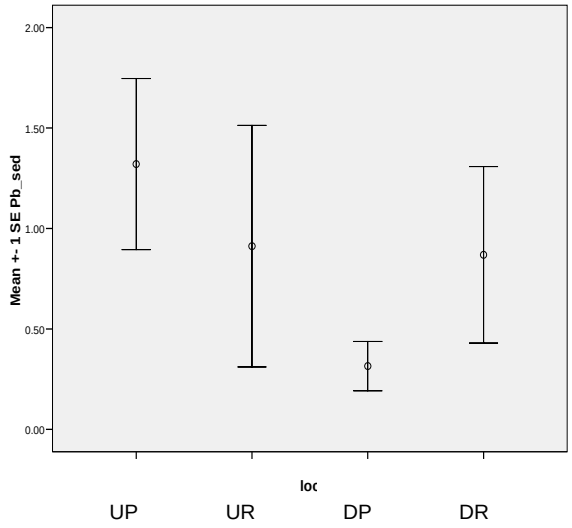


Figure 7.11 Spatial changes in the mean (±1 SE, n=8) lead concentration in sediments (mg/g) upstream (U) and downstream (D) of Cwm Rheidol mine discharge in pools (P) and riffles (R)

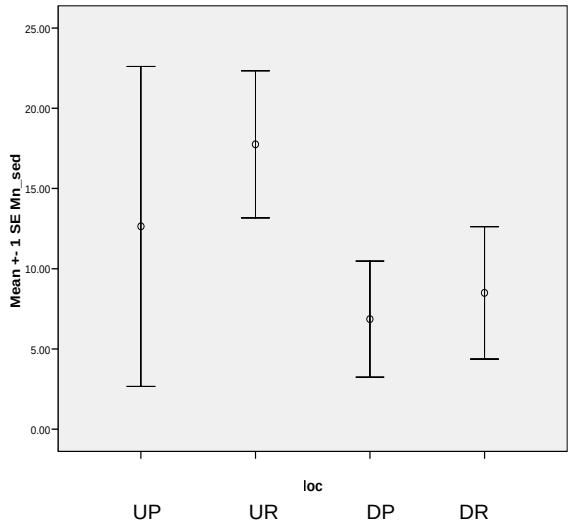


Figure 7.12 Spatial changes in the mean (±1 SE, n=8) manganese concentration in sediments (mg/g) upstream (U) and downstream (D) of Cwm Rheidol mine discharge in pools (P) and riffles (R)

7.3.3 Ecology

One-way ANOVA test detected no significant differences in Ephemeroptera, Plecoptera, Trichoptera and Chironomidae (EPTC) abundance and species richness ($p>0.05$) between upstream and downstream of Cwm Rheidol mine discharge. However, EPTC abundance seem higher downstream from the metal mine discharge than at the upstream sites (Figure 7.13), whereas only species richness in downstream riffles seem to be higher than in any other site (Figure 7.14).

No differences in EPTC abundance and species richness was detected between pools and riffles ($p>0.05$). However, Figures 7.13 and 7.14 seem to suggest that riffles have higher macroinvertebrate abundance and species richness than pools downstream from the mine discharge, whereas upstream, the number of macroinvertebrates in both mesohabitat types is more similar. Species richness appears to be always higher in riffles than in pools, both upstream and downstream from Cwm Rheidol mine discharge.

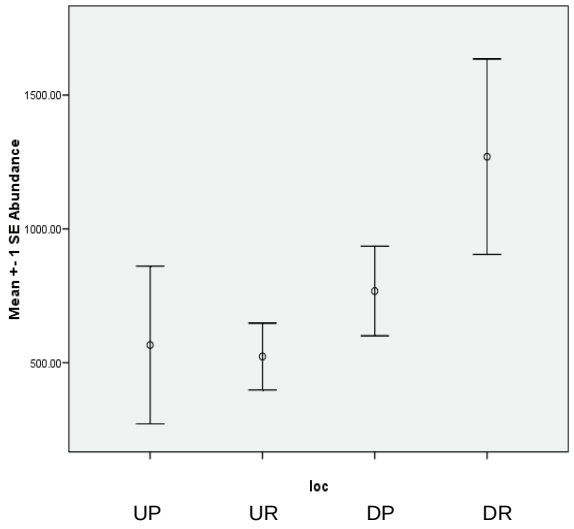


Figure 7.13 Spatial changes in the mean (±1 SE, n=8) abundance of Ephemeroptera, Plecoptera, Trichoptera and Chironomidae in Surber samples upstream (U) and downstream (D) of Cwm Rheidol mine discharge in pools (P) and riffles (R)

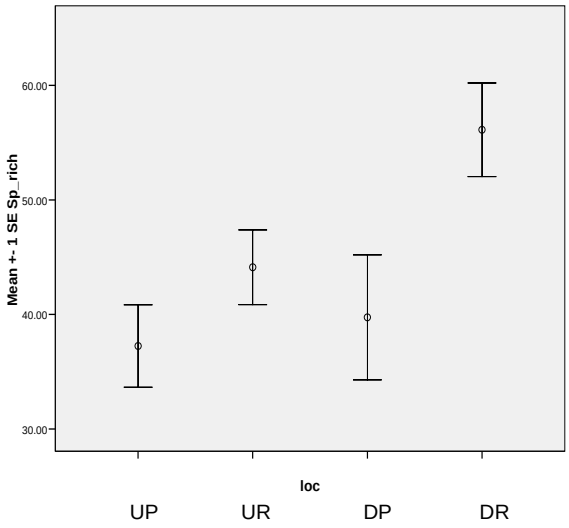


Figure 7.14 Spatial changes in the mean (±1 SE, n=8) species richness of Ephemeroptera, Plecoptera, Trichoptera and Chironomidae in Surber samples upstream (U) and downstream (D) of Cwm Rheidol mine discharge in pools (P) and riffles (R)

7.4 Discussion

7.4.1 Afon Rheidol chemistry around Cwm Rheidol mine

Afon Rheidol water chemistry does not appear to suffer dramatic changes downstream from Cwm Rheidol mine discharge. Only sulphate (in Summer 2006 and Spring 2007), zinc (in Spring 2007) and iron (in Autumn 2006 and Winter 2007) seem to increase at the downstream sites, suggesting that the mine discharge is loaded with sphalerite (ZnS) and pyrite (FeS_2) weathering products (zinc, iron and sulphates) (Equations 7.1 and 7.5). This agrees with the fact that sphalerite was the main ore worked in Cwm Rheidol, and this was rich in pyrite (Bick, 1976). However, iron is always below 1ppm and zinc below 120ppb, which are the EQS (Environmental Quality Standard) published in the Environment Agency Water Framework Directive programme (Chapter 1, Section 1.2). Additionally, zinc and sulphate concentrations (12-46ppb Zn, 4.5-5.6ppm sulphate) are much lower than those recorded in other streams polluted by metal mine drainage in Mid Wales studied in Chapter 3 (64-943ppb Zn, 5.8-10.9ppm sulphate).

Major cation and anion (except sulphate) concentrations in the stream water appear to be constant along the study stretch, suggesting that there is no major input of these ions from the mine or agriculture in the area. In other streams polluted by metal mine drainage studied in this project, nitrate concentrations significantly increased at the downstream sites (Chapter 3), probably due to agricultural input, and this appeared to determine macroinvertebrate community structure (Chapter 4) and grazer abundance (Chapter 5). Agriculture is limited at Cwm Rheidol, thus,

nutrient enrichment seems not to be a problem in the Afon Rheidol. Similarly, SS and river discharge do not present any pattern at the study site.

Conductivity and TDS (total dissolved solids) appear to present a slight increase at the downstream sites in Summer 2006 and Spring 2007. As both variables represent solutes dissolved in the water, this is probably due to an increase in sulphate recorded in the same seasons downstream from Cwm Rheidol mine discharge.

Spoil heaps are common features in mining landscapes and they are formed by the waste material produced during mining activities. Even though it is not economically viable to extract the mineral from waste material, this is still present in the fragments of rock. In spoil heaps, mineral containing trace metals are exposed to the atmosphere, therefore they are constantly being weathered. Spoil heap runoff, loaded with heavy metals and acidity, can impact on receiving water bodies in a similar manner than underground mine discharge. Upstream from Cwm Rheidol mine discharge, there are numerous spoil heaps (Plate 7.1) which may affect the hydrochemistry of the stream. There are no spoil heaps around the downstream sites.

During pyrite weathering acidity is produced, and ochre is formed as a final product of the oxidation process (Equations 7.1-7.3). Orange stains (probably ochre deposits) observed in the spoil heaps at Cwm Rheidol (Plate 7.1) suggest that pyrite weathering is taking place in them. This could also explain the gradual decrease in pH (by 0.5-1 unit) observed along the study stretch, including the upstream sites. This is in agreement with Fuge et al. (1991) study where spoil heap runoff at Cwm Rheidol was seen to present very low pH (2.6-2.7). However, Jones

(1949) suggested in his survey of Afon Rheidol that water mild acidity (pH=5.8-6.4) could also be due to organic acids derived from the peaty soils found in the catchment.

Trace metal concentrations in sediments collected downstream from Cwm Rheidol mine discharge do not seem to significantly vary from those recorded in sediments collected upstream. This is probably due to an input of metals from spoil heap runoff at the upstream sites, which is in agreement with Fuge et al. (1991) study where Cwm Rheidol spoil heap drainage was seen to have high trace metal concentrations (577-978ppm Zn, 1471-3590ppb Cd, 1200-9350ppb Cu, 1950-2430ppb Ni and 955-1870ppb Pb).

Zinc concentrations in Afon Rheidol sediments (1800-12090mg/kg) at the upstream sites are higher than those recorded in sediments from streams polluted by other metal mines in the study area (1900-6400mg/kg) (Chapter 3), and greatly exceed the Threshold Effect Level (TEL) (12.3mg/kg) and Predicted Effect Level (PEL) (315 mg/kg) recommended by the EA (Hudson-Edwards et al. 2008) (Chapter 1). Lead is lower at Afon Rheidol (460-1900mg/kg) than at the other streams (7900-62700mg/kg) (Chapter 3), although it also exceeds the TEL (35mg/kg) and PEL (91.3mg/kg) recommended by the EA (Hudson-Edwards et al. 2008) (Chapter 1). Thus, high zinc concentrations in river bed sediments appear to be the greatest impact of Cwm Rheidol mine pollution when compared to other streams impacted by metal mine drainage in the area, and both zinc and lead exceed the recommended guidelines. As zinc concentrations are high both upstream and downstream, its origin is probably linked to spoil heap run off rather than/as well as water discharge from underground workings. In Chapter 5 it has been suggested that zinc concentration

in sediments, cause negative impacts on benthic predators, hence Afon Rheidol sediments could pose a threat for macroinvertebrate communities inhabiting in them.

No significant differences have been found between metal concentrations in pools and riffles. This seems to contradict Ladd et al. (1998) work where trace metal concentrations were seen to be significantly higher in pools than in riffles.

7.4.2 Afon Rheidol ecology

No significant differences were recorded between macroinvertebrate abundance and species richness upstream and downstream from Cwm Rheidol mine discharge. This could be due to a negative effect of high zinc concentrations recorded in sediments collected upstream from Cwm Rheidol mine discharge, suggesting that spoil heap runoff may be the major process affecting Afon Rheidol ecology. Thus, spoil heap runoff impact must be taken into consideration for Cwm Rheidol mine pollution remediation.

Shannon (2006) reports an increase in salmonid fry abundance downstream from Cwm Rheidol mine discharge, which is attributed to fish acclimatising to high trace metal concentrations in the water. The present study suggests that sediments upstream from Cwm Rheidol mine discharge are severely contaminated, which may be translated into poor benthic community inhabiting them and thus smaller fish populations. However, salmonid populations may recover at the downstream sites due to a bottom up effect in the Afon Rheidol community: sediments containing lower trace metal concentrations can hold more abundant macroinvertebrate

assemblages, which may sustain a bigger fish population. More sampling in Afon Rheidol, reaching further downstream and upstream than the present study, should be undertaken in order to evaluate the hypothesis that sediments, macroinvertebrates and fish recover downstream from Cwm Rheidol mine discharge, where there is no input from spoil heap runoff.

Several studies have found that macroinvertebrate assemblages inhabiting riffles are more abundant and diverse than those inhabiting pools (Brown and Brussock, 1991, Boyero and Bosch, 2004, Costa and Melo, 2008). However, this is not the case in the Afon Rheidol stretch studied, as no significant differences were observed between macroinvertebrate assemblages inhabiting riffles and pools.

7.5 Conclusion

Spoil heaps are exclusively located upstream from Cwm Rheidol mine discharge, and have been found to load runoff with trace metals and acidity (Fuge et al., 1991). Spoil heap runoff may be impacting Afon Rheidol upstream from the mine discharge, explaining the lack of significant differences between trace metal concentrations in sediments and macroinvertebrate assemblages sampled upstream and downstream from the mine discharge. For a better understanding of Cwm Rheidol mine effect on Afon Rheidol, control sampling points should be located further upstream than in the present study, however this was not possible due to difficult access. Thus, the findings of this survey are limited.

No significant differences between pool and riffle macroinvertebrate assemblages were observed in this study. Additionally, sediments collected from pools and riffles did not present significant differences in trace metal concentrations, contradicting Ladd et al. (1998) study, which found that pool sediments had higher metal concentrations than riffle sediments.

In general, Cwm Rheidol mine discharge appears to exert a low impact on stream water chemistry in comparison to other metal mines located in Mid Wales studied in previous chapters, as zinc concentrations are lower (12-46ppb Zn in Afon Rheidol and 64-943ppb Zn in other streams) and both zinc and iron concentrations are always below the EQS of 1ppm for iron and 120ppb for zinc. However, zinc concentrations in sediments upstream from Cwm Rheidol mine discharge (1800-12090mg/kg Zn) appear to be higher than in the other impacted streams (1900-6400mg/kg Zn), and together with lead (460-1900mg/kg Pb) greatly exceed the TEL (12.3mg/kg Zn and 35mg/kg Pb) and PEL (315mg/kg Zn and 91.3mg/kg Pb) suggested by the EA. Thus,

sediment contamination with heavy metals may be the major impact caused by Cwm Rheidol mine drainage. Trace metal concentration in sediment has been seen to impact on benthic predators (Chapter 5), therefore upstream sediments may pose a threat for invertebrate communities inhabiting in them.

8 CONCLUSIONS

8.1 The impact of mine drainage on aquatic communities

This thesis investigated the impact of coal and metal mine drainage on the chemistry (Chapter 3) and ecology (Chapter 4 and 5) of receiving streams. The main objective was to discern the relative effect of pollutants in the water and bed sediment, in order to improve our understanding of how macroinvertebrates are affected by mine drainage and consider the implications of this for improving remediation techniques.

Streams impacted by coal mine drainage were characterised by an increase in iron (up to 772ppb), manganese (up to 700ppb) and sulphate (up to 118ppm) concentrations in water, a decrease in pH (although always circumneutral), high suspended solids (ochre flocs) (up to 8ppm) and high iron concentration in sediments (up to 16000mg/kg) (Chapter 3). From all these components, only sulphate and manganese exceeded the environmental quality standards (EQS) derived from the water framework directive (WFD) of 87.5ppm SO_4^{2-} and 17.5ppb Mn^{2+}. Benthic species richness and abundance were also reduced downstream from the coal mines (Chapter 4). The negative impact of coal mine drainage on the structure of the community inhabiting receiving streams appeared to be mainly due to ochre deposition covering the stream bed and smothering macroinvertebrates (Chapter 4). Further study suggested that different functional groups respond in a different manner to coal mine drainage components: grazers were mainly affected by suspended solids

(SS), collector-gatherers and predators by pH, shredders by iron concentration in sediments and filter-feeders by iron concentration in the water column (Chapter 5). Hence, it has been suggested that both water (pH and iron concentration) and sediment (SS and iron concentration) related variables influence macroinvertebrates inhabiting streams impacted by coal mine drainage.

Streams impacted by metal mine drainage were characterised by increases in concentration of zinc (up to 1450ppb), sulphate (up to 10ppm) and nitrate (up to 3.77ppm, probably originating in the agricultural land surrounding the sites) in water, and sediments loaded with zinc (6440mg/kg), lead (62650mg/kg) and copper (1460mg/kg) (Chapter 3). Zinc concentration in water exceeded the EQS of 120ppb, and zinc and lead concentration in sediments exceeded the TEL (Threshold Effect Level) of 12.3mg/kg Zn^{2+} and 35mg/kg Pb^{2+} suggested by the EA. Benthic species diversity and evenness were reduced downstream from the metal mines (Chapter 4). Structural analysis of streams impacted by metal mine drainage highlighted nitrate as the main factor conditioning macroinvertebrate assemblages, whereas functional studies demonstrated that trace metal concentration in water (shredders, collector-gatherers and filter-feeders) and sediments (predators) was an important factor impacting on stream ecology. Nitrate concentrations seemed to impact on grazer macroinvertebrates, suggesting that this negative effect might be linked to food availability or quality. Nitrate has been seen to enhance copper toxicity for diatoms (Rijstenbil et al., 1998) and similar interactions with other trace metals could occur in the study streams.

The present study suggested that sediment monitoring and remediation is a key aspect in mine drainage pollution of freshwater ecosystems, as the functional structure of the study streams appear to be affected by trace metal concentration in sediments. However, the WFD does not include metal concentration guidelines for sediments, neither has the UK set mandatory standards for them, and sediments are not currently being routinely monitored or remediated in the UK (Hudson-Edwards et al., 2008). The EA is currently developing a programme to assess metal contamination of river sediments suggesting concentration guidelines for some trace metals (arsenic, zinc, lead, cadmium and copper), although other metals such as iron and manganese are still not represented. Streams impacted by metal mine drainage in the present study greatly exceed the guidelines for zinc and lead concentrations in sediments.

8.2 Implications for mine water remediation

Both types of mine drainage appear to affect the chemical and ecological quality of receiving streams, and threaten the fulfilment of the WFD requirements of good ecological status by 2015. In the UK, the EA and the CA are working together to remediate mine drainage. Chapter 7 studied the recovery of a stream (Smithy Brook) severely impacted by coal mine drainage after remediation was initiated. The constructed engineered wetland, successfully remediated mine water removing trace metals and suspended solids and increasing pH and DO. However, the remediation scheme seemed to fail to improve the electrolyte status of the water and stream sediment quality. As a result,

the benthic community in Smithy Brook appeared to have a poor recovery. After 3 months of water improvement, Chironomidae seemed to increase in abundance, followed by re-colonisation of *Baetis rhodani* a month later. However, there seemed to be no re-colonisation by other organisms. The study suggested that treating mine water may not be enough to gain ecological recovery of a stream within a time scale of 6 months. The key recommendations derived from this study for the improvement of coal mine drainage remediation to seek ecological recovery of impacted streams are:

(a) Modify treatment schemes to remediate electrolyte status of the water in addition to the aspects already being successfully removed such as iron, SS and acidity;

(b) Actively remediate contaminated sediment, as this has not been proven to be induced by water treatment;

(c) Ecological recovery must be monitored after treatment is initiated to ensure success of remediative techniques, as chemical recovery of stream water seems not to be a valid indicator of stream recovery.

Engineered wetlands are currently being constructed in the UK to treat drainage from several coal mines, with forty six schemes constructed by the CA to date, (Johnston and Rolley, 2008). However, passive techniques for metal mine drainage treatment are still under development, with only one full-scale wetland built in the UK: Wheal Jane in Cornwall (Johnston and Rolley, 2008). Currently, an EU funded project is being managed by EA Wales in conjunction with the Welsh Assembly and the CA to remediate Cwm Rheidol mine discharge and improve the ecological status of Afon Rheidol (Edwards and

Potter, 2007). Chapter 8 consisted of a preliminary study of Afon Rheidol to determine its ecological and chemical quality in relation to the mine discharge. The study did not observe significant differences between upstream and downstream macroinvertebrate assemblages, nor chemical quality of the stream water and sediment. This fact might be linked to the input from spoil heap runoff in the upstream sites, suggesting that this might be a factor to take into consideration for the design of the remediation scheme. However, this could not be proven and the impact of pollution originating from spoil heaps on stream biota needs further study.

The key recommendations derived from this study to be considered in the development of metal mine drainage remediation techniques are:

(a) Reduce trace metal input to the streams. In the study mines, only zinc exceeded the EQS, however both zinc and lead exceeded the recommended guidelines for sediment concentration, suggesting that lead is also present in the mine discharge.

(b) Trace metal concentration in stream sediments exceeded the guidelines suggested by the EA, and impacted on the fauna (invertebrate predators) inhabiting the streams. Therefore, sediment remediation must also be priority aspect in metal mine drainage treatment, if this is not seen to recover after water remediation.

(c) Input of trace metals and acidity from spoil heap run off has been proven (Fuge et al., 1991). Findings on this subject from Chapter 8 are not conclusive, but spoil heaps might need consideration to ensure success in mine drainage remediation.

8.3 Degree of objectives fulfilment

The aims and objectives of this study have been presented in the Introduction (Section 1.4) and in the relevant sections in each chapter, this section summarises the degree of achievement of each objective. The main discussion resulting from each question is more developed in each chapter and summarise in the other sections of this Conclusions chapter.

1. *Determine the impact of coal and metal mine drainage on stream water, sediment and ecology*. This overarching aim was approached through several more specific objectives:

a) *Determine the environmental variables (water and sediments) affected by mine drainage pollution which will be used within the ecological study (Chapter 3, Sections 3.4.1.1 and 3.4.2.1).* This was determined through the physic-chemical study of eight Welsh streams receiving coal and metal mine drainage. Coal mine drainage was seen to significantly increase iron, manganese, sulphate, acidity and suspended solids in water, and increase iron concentration in sediments, whereas metal mine drainage was seen to increase zinc, sulphate and nitrate in water, and zinc, lead and copper in sediments.

b) *Establish whether metals are predominantly found in the water or sediment phase within different impacted streams (Chapter 3, Sections 3.4.1.2 and 3.4.2.1).* This objective was partially achieved due to the limitations in the data set forming this thesis, and needs further more detailed chemical study of freshwater environments polluted by abandoned mine drainage. Nevertheless, trace metals proved to be transferred from the water column into the sediment fraction in the river through precipitation (in the case of iron) and adsorption mechanisms (in the case of lead, zinc and copper).

c) *Identify the key differences in water chemistry between coal and metal mine drainage (Chapter 3, Section 3.5).* The main differences observed in rivers polluted by coal and metal mine drainage are due to the nature of the worked material. Coal mines load receiving streams mainly with iron, which precipitates as ochre and covers the stream bed with orange solids. On the other hand, metal mines main impact is linked to dissolution of trace metals in water, which adsorb to the sediments, but there is no associated precipitate.

d) *Determine if abandoned mine drainage impacts on the structure of the aquatic community in receiving streams (Chapter 4, Sections 4.5.1 and 4.5.2).* Coal mine drainage was seen to significantly reduce benthic species richness and abundance, and metal mine drainage reduced benthic species diversity and evenness.

e) *Determine if abandoned mine drainage negatively affects the function of the aquatic community in receiving streams (energy inputs and functional structure) (Chapter 5, Sections 5.5.1 and 5.5.2).* The present study suggests that the stream ecosystem function is negatively impacted by coal and metal mine drainage.

2. *Determine the relationship between specific environmental variables derived from coal and metal mine drainage and aquatic communities.* Statistical analysis relating environmental and ecological variables measured in eight Welsh streams impacted by coal and metal mine drainage was used to explore this aim.

a) *Assess the relative importance of water related variables and sediment related variables in predicting macroinvertebrate community structure at the sampled streams (Chapter 4, Sections 4.5.1 and 4.5.2).* In coal mine impacted streams, sediment related variables seemed to be the main drivers impacting on assemblage structure, whereas in metal mine impacted streams, water related variables were more relevant.

b) *Relate environmental variables to changes in benthos composition (Chapter 4, Sections 4.5.1 and 4.5.2).* In streams impacted by coal mine drainage, suspended sediment seemed to be the most important variable in explaining a negative response to pollution. In metal mine polluted streams, nitrate appeared to be the main driver in the impact on freshwater communities.

c) *Detect differences in benthos response to coal and metal mine drainage pollution (Chapter 4, Section 4.6)*. Macroinvertebrate assemblage in streams polluted by coal mine drainage seemed to mainly respond to the physical effect of ochre sedimentation, whereas the main impacting variable in metal mine drainage was nitrate concentration in waters, possibly linked to interactions with trace metals and their toxicity.

d) *Relate environmental variables to functional changes in the benthic community (Chapter 5, Sections 5.5.1 and 5.5.2)*. Different functional groups responded different to the variables altered by mine pollution. In streams impacted by coal mine drainage, grazers respond mainly to suspended sediments, collector-gatherers and predators to pH, shredders to iron concentration in sediments and filter-feeders to iron concentration in water. In streams impacted by metal mine drainage, shredders, collector-gatherers and filter-feeders respond mainly to trace metal concentration in water, predators to trace metal concentration in sediments, and grazers to nitrate concentrations in water.

e) *Detect differences in ecosystem functional response to coal and metal mine drainage pollution (Chapter 5, Section 5.6)*. In streams impacted by coal mine drainage the impact on the ecosystem function was mainly linked to iron and related variables (pH and suspended sediments). In streams polluted by metal mine drainage, metal concentration in water and sediment seemed to be the drivers for a negative impact on ecosystem function.

3. *Investigate the ecological and chemical response of a polluted stream to mine water remediation (Chapter 6)*. This aim was investigated with the intense study of Smithy Brook, a stream historically polluted by coal mine drainage which is now being remediated.

a) *Establish if there is water and sediment quality recovery after the remediation scheme becomes operational (Section 6.5.1)*. The engineered wetland constructed by the Coal Authority to treat Pemberton coal mine drainage before entering Smithy Brook proved to successfully remediate mine water removing trace metals, suspended solids, acidity and increasing dissolved oxygen. On the other hand, the remediation scheme seems to fail in improving the electrolyte status of the water and trace metals concentration in the stream sediments.

b) *Determine response times and spatial variations for the different environmental variables (water and sediment) related to mine pollution (Sections 6.5.2 and 6.5.3)*. This objective was only partially fulfilled due to the time limitations of the study. Trace metal concentration, pH, dissolved oxygen and suspended solids present an immediate response to water remediation. However, trace metal concentration may need more time to recover from coal mine pollution. More study is needed in this aspect to establish sediment recovery time scales.

c) *Investigate if there is ecological recovery and establish a timescale (Section 6.5.4).* This objective was only partially achieved due to the time limitations of the study. After 3 months of water remediation, Chironomidae appeared to increase in abundance, followed by re-colonisation of *Baetis rhodani* a month later. However, longer studies may be required to establish recovery times for other taxonomic groups.

4. *Study of a stream currently receiving metal mine drainage (Cwm Rheidol) prior to the design of a remediation scheme (Chapter 7).* This aim is addressed though the intensive study of Afon Rheidol as it flows through Cwm Rheidol metal mine.

a) *Determine if Cwm Rheidol mine is negatively impacting on Afon Rheidol water, sediment, and ecological quality (Sections 7.4.1 and 7.4.2)*;

b) *Determine if there is spatial recovery as the river flows downstream from the mine discharge (Section 7.4.1 and 7.4.2)*;

c) *Investigate differences in water chemistry, trace metal concentration in sediments and ecology between riffles and pools (Sections 7.4.1 and 7.4.2).*

It was not possible to respond these questions due to the impact of spoil heaps upstream from Cwm Rheidol mine discharge and the impossibility to access sampling sites further upstream.

8.4 Future research

Water toxicity is rarely determined by one type of substance, but interactions between dissolved pollutants are poorly understood (Ivorra et al., 2002). The present study draws attention to the lack of understanding in the interactions between nitrate and trace metals. Nitrate has proved to be a key component on the negative effect observed on macroinvertebrate communities, and although it has previously been found to produce a negative effect on invertebrates in streams impacted by mine drainage (Hirst et al., 2002), the reasons behind this are not fully understood. Nitrate could enhance metal toxicity in diatoms (Rijstenbil et al., 1998), thereby affecting the whole ecosystem from the bottom up; or decrease chironomid oviposition (Stevens et al., 2003) (Chapter 4). Hence, further research in interactions between contaminants present in water bodies is needed for a better understanding of polluted environments, not only in relation to mining activities but also other origins such as industry and urbanisation.

The effect of seasonal and flow variations on the chemistry and ecology of streams impacted by mine drainage were out of the scope of this study. However, other research has suggested that they might be important in controlling mine pollution impact on aquatic communities (Herricks and J., 1972, Herricks and Cairns, 1977). This is especially apparent in relation to spoil heap runoff, which seems to respond to rainfall events (Canovas et al., 2008). Spoil heap runoff has been seen to contain high concentrations of trace metals (Fuge et al., 1991), and their impact on aquatic

communities might equal or exceed that of underground mine water, yet their effect on aquatic communities is not well studied. In Chapter 3-6, the effect of spoil heap runoff on the chemistry and ecology of receiving streams was studied in conjunction with underground mine discharge, by locating downstream sampling points downstream from both spoil heaps and underground mine drainage. However, in Chapter 8, more sampling points were located along Afon Rheidol, and spoil heap impact was separated from that of underground drainage. Nevertheless, the effects of spoil heap runoff on aquatic ecosystems were not conclusive. Hence, more research should be undertaken with spoil heaps, in order to understand their relative importance in the impact of mine pollution on aquatic communities and the seasonal variations in their inputs. This is highly relevant for the design of mine water remediation schemes, as these only treat underground mine water, which may result in failure of the scheme if spoil heaps are present.

Further research is also required to improve schemes treating coal mine drainage in order to remediate the electrolyte status of the water (especially sulphate) as well as trace metal concentration and acidity, as this may be impacting on aquatic ecosystems (Chapter 7). Additionally, longer recovery studies may be needed to establish if ecology and/or sediments require more than 6 months to recover once water is clean, or if active measures to clean sediments are necessary. If ecology is seen to recover after 6 months or after actively cleaning the sediments, monitoring of the stream community may be required to: (a) establish if communities are fully functional (e.g. looking at functional measures such as decomposition and production); and (b) compare the stream community to reference sites (such as upstream from the mine if adequate) to identify factors which

may prevent fully re-colonisation of macroinvertebrates, such as residual toxicity in sediments or effects of community closure (Chapter 7).

The present study has demonstrated that sediment quality impacts on macroinvertebrate communities inhabiting streams polluted by mine drainage. However, sediment toxicity in mine pollution should be further studied in conjunction with the development of quality guidelines. From this research, and following the work the author undertook in the Centre of Environmental Stress and Adaptation Research (CESAR) at the University of Melbourne (Australia) as part of the scheme Universitas 21, contaminated sediment toxicity on aquatic communities could be studied from a different angle to fieldwork surveys: via controlled microcosm experiments. This technique consists in placing polluted sediments in tanks containing clean water, locate them nearby an unpolluted water body to allow them to be colonised by adult oviposition, and monitor Chironomidae emergence. This method establishes a semi-controlled environment, isolated from effects of other extraneous factors, which allows determining the effect of sediment toxicity or avoidance by ovipositor adults (Pettigrove and Hoffmann, 2005). Previous work has been undertaken with microcosm experiments to study urban streams, which receive run off typically composed by heavy metals, polyaromatic hydrocarbons and pesticides (e.g. Pettigrove and Hoffmann, 2003). Microcosm experiments with sediments from streams polluted by mine drainage would provide crucial information in discerning the relative importance of water and sediment related variables in mine drainage pollution of fresh water ecosystems.

APPENDIX A. CHEMICAL DATA FROM THE STUDY SITES

In this appendix several tables with the raw chemical data from the study sites are presented. Firstly, coal mine drainage impacted streams are displayed, followed by metal mine drainage impacted ones. Up and downstream samples for each site are displayed. Four samples a year were taken in each stream, corresponding to the four seasons.

Cells that are found blank correspond to missing samples.

Sites are displayed with codes, the first one or two letters represent the site name, table A.1 shows the correspondence between each site and its first letter of the code. Following, U or D correspond to upstream and downstream. The last one or two letter in the code represent the season (S: summer, A: Autumn, W: Winter and SP: Spring).

Hence AUA corresponds to Aberbaiden Upstream in Autumn.

Table A.1 Sampling sites codes

Mine type	Site	Code
Coal mines	Aberbaiden	A
	Cwm Gros	CG
	Clyne	C
	NantyFyllon	NF
Metal mines	Dylife	D
	Frongoch	F
	Cwm Symlog	CS
	Cwm Ystwyth	CY

Streams impacted by coal mine drainage

Table A.2 Water chemistry in sites impacted by coal mine drainage

	ppm				ppb				ppm			
	Chloride	**Nitrate**	**Sulphate**	**Phosphate**	**Mn**	**Fe**	**Ni**	**Zn**	**Na**	**Mg**	**K**	**Ca**
AUS	16.93	1.39	22.42	0.14	0.00	40.00	0.00	0.00	7.16	4.23	1.15	11.77
ADS	20.57	0.97	273.56	0.16	1720.00	620.00	0.00	0.00	25.49	26.25	5.32	69.68
NFUS	9.38	0.86	15.93	0.34	0.00	0.00	0.00	0.00	5.45	4.30	1.32	7.13
NF/S	11.09	0.48	50.09	0.08	170.00	250.00	0.00	0.00	6.09	9.80	4.58	23.09
CUS	21.14	0.81	78.73	0.16	0.00	20.00	0.00	0.00	11.79	18.55	4.84	63.64
CDS	19.56	1.01	70.60	0.16	220.00	0.00	0.00	0.00	11.38	17.69	4.41	63.34
CGUS	8.69	0.57	13.85	0.21	0.00	0.00	0.00	0.00	4.36	2.12	0.69	8.40
CGDSP	9.32	1.16	80.69	0.22	170.00	1920.00	0.00	0.00	82.60	11.88	10.10	27.20
AUA	35.79	2.54	48.99	0.06	0.00	150.00	0.00	0.00	7.79	2.73	1.18	8.02
ADA	16.66	3.31	35.33	0.04	0.00	410.00	0.00	0.00	13.26	10.94	2.86	26.98
NFUA	15.79	4.51	26.24	0.00	0.00	70.00	0.00	0.00	5.06	2.89	0.86	4.09
NFDA	78.91	3.82	53.42	0.20	0.00	630.00	0.00	0.00	5.40	6.61	2.66	12.13
CUA	20.34	3.34	112.14	0.29	0.00	400.00	0.00	0.00	10.38	12.68	5.68	44.97
CDA	19.87	3.70	117.57	0.07	0.00	330.00	0.00	0.00	10.87	12.74	4.39	43.73
CGUA	6.39	2.10	48.85	0.74	0.00	160.00	0.00	0.00	4.46	1.67	0.57	6.27
CGDA	66.46	2.28	92.27	0.38	0.00	420.00	0.00	0.00	76.32	9.16	9.16	16.43

Table A.2 Continuation Water chemistry in sites impacted by coal mine drainage

	ppm				ppb				ppm			
	Chloride	**Nitrate**	**Sulphate**	**Phosphate**	**Mn**	**Fe**	**Ni**	**Zn**	**Na**	**Mg**	**K**	**Ca**
AUW	18.70	7.38	12.79	0.01	30.00	70.00	0.00	0.00	9.30	2.84	1.30	5.60
ADW	19.14	6.69	66.17	0.02	440.00	830.00	0.00	10.00	16.40	10.53	2.87	17.07
NFUW	10.96	3.11	12.43	0.01	20.00	0.00	0.00	8.00	6.15	2.83	0.86	2.66
NFDW	11.07	2.83	18.26	0.00	60.00	180.00	0.00	8.00	6.27	4.75	1.56	5.04
CUW	20.76	9.18	37.08	0.00	0.03	0.00	0.00	0.02	12.34	11.45	4.20	30.11
CDW	20.03	6.59	32.56	0.01	29.62	0.00	0.00	19.09	8.51	9.21	3.17	25.21
CGUW	8.97	1.20	4.89	0.01	20.00	60.00	0.00	5.00	5.84	1.87	0.70	4.36
CGDW	8.86	1.11	24.97	0.01	150.00	460.00	0.00	9.00	25.55	4.72	3.33	6.58
AUSP	17.89	5.51	15.27	0.10	70.00	150.00	0.00	0.00	8.89	2.91	1.34	5.50
ADSP	15.72	4.05	97.31	0.23	620.00	1200.00	9.00	10.00	14.30	10.22	2.71	15.25
NFUSP	8.66	0.73	8.48	0.26	120.00	360.00	0.00	0.00	6.16	5.68	1.95	6.69
NFDSP	7.65	0.00	67.30	0.21								
CUSP	18.94	1.78	62.67	0.23	240.00	340.00	0.00	0.00	10.48	9.14	4.66	22.71
CDSP	18.06	1.14	54.06	0.16	380.00	500.00	0.00	0.00	10.45	9.40	3.39	22.02
CGUSP	7.64	0.00	6.22	0.19	30.00	130.00	0.00	0.00	4.91	1.53	0.60	4.18
CGDSP	5.78	0.00	59.58	0.18	110.00	290.00	0.00	0.00	18.47	3.66	2.51	6.25

Table A.3 Sediment chemistry (mg/g) in sties impacted by coal mine drainage

	Mn Sed	Ni Sed	Fe Sed	Cu Sed	Zn Sed	Pb sed	Cd Sed
AUS	8.35	0.00	58.24	0.02	0.35	0.01	0.00
ADS	6.96	0.00	45.08	0.11	0.91	0.01	0.00
NFUS	3.42	0.02	33.64	0.05	0.51	0.01	0.00
NF/S	8.06	0.00	104.37	0.00	0.42	0.01	0.00
CUS							
CDS							
CGUS							
CGDSP							
AUA	6.27	0.35	75.68	0.10	0.85	0.03	0.00
ADA	9.26	0.20	61.73	0.17	0.42	0.02	0.00
NFUA	2.20	0.41	30.12	0.20	1.06	0.04	0.00
NFDA	0.96	0.42	45.48	0.27	1.37	0.57	0.00
CUA	3.32	0.14	75.30	0.07	0.47	0.01	0.00
CDA	3.29	0.14	110.43	0.08	0.25	0.01	0.00
CGUA	1.51	0.08	63.91	0.06	0.41	0.01	0.00
CGDA	1.37	0.12	69.30	0.08	0.49	0.01	0.00
AUW	4.09	0.06	23.42	0.02	0.26	0.06	0.00
ADW	5.61	0.00	413.39	0.20	1.01	0.08	0.00
NFUW	5.29	0.17	30.28	0.16	0.84	0.03	0.01
NFDW	19.58	0.20	1.88	0.27	0.91	0.03	0.00
CUW	5.12	0.08	48.67	0.04	0.16	0.00	0.00
CDW	10.77	0.08	4.17	0.03	0.16	0.00	0.00
CGUW							
CGDW	1.42	0.38	0.00	0.04	0.26	0.00	0.00

Table A.3 Continuation Sediment chemistry (mg/g) in sties impacted by coal mine drainage

	Mn Sed	Ni Sed	Fe Sed	Cu Sed	Zn Sed	Pb sed	Cd Sed
AUSP	5.57	0.20	46.69	0.65	0.59	0.03	0.00
ADSP	6.63	0.24	118.97	0.22	0.63	0.02	0.00
NFUSP	5.93	0.53	24.01	0.35	0.56	0.07	0.00
NFDSP	0.00	0.00	0.01	0.00	0.00	0.00	0.00
CUSP	2.32	0.13	39.62	0.16	0.23	0.02	0.00
CDSP							0.00
CGUSP	2.27	0.10	49.39	0.14	0.38	0.02	0.00
CGDSP	3.92	0.13	67.88	0.14	0.44	0.03	0.00

Table A.4 Other hydrochemical variables in streams impacted by coal mine drainage

	ph	COND (µS)	T (ºC)	TDS (ppm)	Flow (m3/s)	SS (mg/l)	Width (m)	DO (%)
AUS	7.30	169.60	13.80	115.30	4.77	1.51	3.76	104.00
ADS	7.11	674.20	13.40	475.70	11.00	10.37	2.90	107.00
NFUS	6.79	106.60	14.30	71.87	2.31	0.00	1.45	120.00
NF/S	6.62	261.70	12.80	179.80	1.95	4.23	1.25	123.00
CUS	7.73	489.10	13.20	343.60	1.60	1.17	2.00	98.00
CDS	7.28	492.70	13.20	346.30	3.24	2.41	2.30	99.00
CGUS	6.88	109.80	16.50	73.78	5.64	2.78	2.35	101.00
CGDSP	6.81	553.80	15.60	387.80	6.84	6.67	1.60	74.00
AUA	7.06	129.60	10.10	86.51	31.97	1.92	3.76	99.70
ADA	7.01	358.30	10.00	246.70	37.05	3.65	2.90	102.50
NFUA	6.73	93.84	9.50	62.78	2.46	0.00	1.45	105.80
NFDA	6.63	183.60	10.70	123.80	1.21	1.18	1.25	99.50
CUA	7.31	419.80	9.00	291.10	1.57	2.69	2.00	91.20

Table A.4 Continuation Other hydrochemical variables in streams impacted by coal mine drainage

	ph	COND (µS)	T (ºC)	TDS (ppm)	Flow (m3/s)	SS (mg/l)	Width (m)	DO (%)
CDA	7.01	405.30	9.30	280.70	3.94	4.04	2.30	87.20
CGUA	6.06	81.66	4.40	55.25	7.38	2.64	2.35	93.40
CGDA	6.58	484.60	7.20	338.90	12.67	7.55	1.60	73.20
AUW	7.24	125.30	9.90	85.91	11.50	2.22	3.76	97.80
ADW	7.00	281.70	10.10	197.60	11.89	5.56	2.90	97.90
NFUW	7.96	81.93	9.40	59.36	70.61	0.20	1.45	98.80
NFDW	7.47	122.60	9.50	84.14	95.92	2.41	1.25	96.40
CUW	7.21	271.20	9.70	190.00	15.66	5.88	2.00	93.80
CDW	7.04	281.50	10.20	197.40	17.73	8.15	2.30	87.20
CGUW	6.74	68.84	7.00	47.67	**82.48**	4.81	2.35	91.60
CGDW	6.64	213.50	7.40	150.10	64.48	4.81	1.60	82.60
AUSP	7.43	113.90	11.80	77.22	17.21	7.06	3.76	102.90
ADSP	7.17	291.40	11.80	202.00	26.76	9.81	2.90	102.70
NFUSP	7.28	80.43	10.80	54.73	5.05	1.96	1.45	104.10
NFDSP	6.95	138.90	10.20	94.42	5.05	1.96	1.25	100.90
CUSP	7.34	295.00	12.40	204.20	3.59	9.23	2.00	99.80
CDSP	7.09	301.90	11.90	209.30	5.26	12.24	2.30	93.30
CGUSP	7.18	74.00	12.00	50.24	36.42	4.23	2.35	93.70
CGDSP	7.03	161.40	12.40	109.70	28.18	11.73	1.60	89.70

Metal mine impacted streams

Table A.5 Water chemsitry in streams impacted by metal mine drainage

	ppm				ppb					ppm			
	Chloride	Nitrate	Sulphate	Phosphate	Mn	Fe	Zn	Cd	Pb	Na	Mg	K	Ca
DUS	6.24	2.04	2.00	0.38	0.00	280.00	0.00	40.00	90.00	4.18	1.16	0.59	3.28
DDS	11.42	3.03	16.02	0.38	0.00	0.00	2150.00	70.00	350.00	4.89	1.73	0.88	5.36
FUS	10.37	0.82	7.53	0.36	0.00	0.00	2.00	0.00	0.00	8.56	3.01	0.62	12.46
FDS	11.81	1.89	14.23	0.36	0.00	0.00	2800.00	0.00	0.00	6.56	1.82	0.67	7.57
CYUS	5.21	0.75	4.84	0.36	0.00	0.00	0.00	0.00	0.00	3.70	0.87	0.37	2.10
CYDS	10.58	2.26	9.30	0.40	0.00	0.00	870.00	0.00	0.00	5.18	1.58	0.42	4.94
CSUS	11.82	0.73	4.82	0.38	0.00	0.00	0.00	0.00	0.00	7.10	1.26	0.32	3.19
CSDS	11.18	3.24	10.44	0.36	0.00	0.00	0.00	0.00	0.00	8.29	3.57	0.92	12.25
DUA	6.51	6.04	3.21	0.00	0.00	90.00	0.00	0.00	0.00	3.52	0.83	0.39	2.61
DDA	6.46	4.84	5.50	0.01	0.00	90.00	410.00	0.00	80.00	4.01	1.06	0.61	2.97
FUA	8.35	3.86	7.43	0.02	0.00	130.00	0.00	0.00	0.00	5.44	1.42	0.57	5.01
FDA	8.17	3.97	9.52	0.01	0.00	100.00	0.00	0.00	70.00	5.32	1.32	0.55	4.80
CYUA	5.67	1.46	3.82	0.02	0.00	150.00	0.00	0.00	0.00	3.37	0.67	0.26	1.15
CYDA	5.70	1.41	5.08	0.02	0.00	130.00	260.00	0.00	0.00	3.55	0.76	0.32	1.34
CSUA	9.55	1.85	6.23	0.02	0.00	90.00	0.00	0.00	0.00	5.57	1.15	0.32	2.24
CSDA	10.17	5.72	9.70	0.00	0.00	80.00	0.00	0.00	0.00	6.24	1.69	0.66	4.85

Table A.5 Continuation Water chemsitry in streams impacted by metal mine drainage

	ppm				ppb					ppm			
	Chloride	Nitrate	Sulphate	Phosphate	Mn	Fe	Zn	Cd	Pb	Na	Mg	K	Ca
DUW	10.86	3.87	3.44	0.25	20.00	30.00	350.00	0.00	100.00	7.47	1.85	0.73	3.26
DDW	11.07	5.24	5.22	0.25	20.00	40.00	10.00	0.00	0.00	3.58	0.76	0.12	1.93
FUW	9.68	3.26	5.23	0.13	10.00	80.00	70.00	0.00	0.00	4.83	1.33	0.48	3.34
FDW	9.86	2.83	6.98	0.20	10.00	50.00	1100.00	0.00	0.03	6.21	1.67	0.58	3.49
CYUW	6.75	0.84	3.12	0.25	40.00	90.00	20.00	0.00	0.00	4.51	0.97	0.36	0.92
CYDW	6.32	0.88	3.03	0.25	40.00	90.00	150.00	0.00	0.01	4.91	1.04	0.44	1.06
CSUW	11.98	1.21	5.50	0.20	20.00	30.00	90.00	0.00	0.01	5.61	1.75	0.28	4.49
CSDW	12.40	3.77	10.87	0.21	70.00	30.00	7.00	0.00	0.00	8.33	1.61	0.46	1.71
DUSP	8.58	0.62	2.63	0.26	20.00	100.00	0.00	0.00	0.00	4.70	1.17	0.16	2.62
DDSP	9.60	1.59	8.00	0.27	10.00	0.00	1200.00	0.00	200.00	7.14	2.16	0.61	4.82
FUSP	9.49	1.09	4.82	0.26	10.00	110.00	70.00	0.00	0.00	7.03	2.38	0.38	5.44
FDSP	9.86	1.50	9.61	0.19	0.00	30.00	1900.00	0.00	100.00	7.57	2.34	0.43	5.46
CYUSP	6.33	0.23	3.05	0.23	0.00	30.00	5.00	0.00	0.00	4.33	0.85	0.20	1.04
CYDSP	6.48	0.22	5.74	0.28	0.00	20.00	360.00	0.00	0.01	4.52	1.17	0.18	1.58
CSUSP	14.56	1.06	5.46	0.19	0.00	0.00	0.00	0.00	0.00	10.49	2.08	0.42	2.66
CSDSP	15.84	2.36	12.47	0.25	10.00	70.00	250.00	0.00	100.00	13.25	4.51	0.84	8.81

Table A.6 Sediment chemistry (mg/g) of streams impacted by metal mine drainage

	MnSed	NiSed	FeSed	CuSed	ZnSed	PbSed	CdSed
DUS	33.42	0.02	64.65	0.05	0.87	0.68	0.05
DDS	0.00	0.00	161.69	1.11	4.07	64.58	0.08
FUS	5.44	0.01	47.83	0.02	1.09	3.31	0.03
FDS	1.07	0.01	51.72	0.09	3.21	11.74	0.04
CYUS	3.49	0.02	93.97	0.00	0.28	0.08	0.05
CYDS	2.17	0.06	48.97	0.02	1.46	2.33	0.03
CSUS	14.28	0.00	58.23	0.06	0.87	26.64	0.03
CSDS	2.96	0.02	100.24	0.13	0.73	12.30	0.03
DUA							
DDA	98.50	0.00	166.41	1.37	6.27	61.39	0.53
FUA	0.01	0.02	77.07	0.02	1.49	3.91	0.04
FDA	86.92	0.00	86.48	0.00	4.20	14.49	0.31
CYUA	0.00	0.00	16.24	0.00	0.40	0.13	0.08
CYDA							
CSUA	0.00	0.04	6.55	0.08	0.56	44.45	0.05
CSDA	175.31	0.00	120.25	0.00	1.35	16.34	0.43
DUW	4.34	0.00	79.00	2.05	14.77	91.37	0.33
DDW							
FUW	2.47	0.00	85.38	0.00	3.65	0.00	0.56
FDW	3.86	0.00	94.35	0.09	6.70	53.80	0.31
CYUW	3.93	0.00	190.34	0.00	1.59	0.00	0.26
CYDW	3.50	0.02	49.18	0.07	1.54	7.51	0.03
CSUW	2.33	0.00	56.40	0.00	1.87	0.00	0.24
CSDW	2.25	0.00	59.38	0.88	1.98	28.86	0.20

Table A.6 Continuation Sediment chemistry (mg/g) of streams impacted by metal mine drainage

	MnSed	NiSed	FeSed	CuSed	ZnSed	PbSed	CdSed
DUSP	12.87	0.00	49.05	0.25	0.89	3.08	0.07
DDSP	0.76	0.00	54.34	1.91	8.98	61.99	0.08
FUSP							
FDSP	2.12	0.00	67.50	0.19	2.48	22.83	0.05
CYUSP	1.33	0.03	45.39	0.01	0.29	0.00	0.02
CYDSP	2.92	0.04	52.64	0.16	4.85	13.77	0.07
CSUSP	2.51	0.01	40.72	0.02	0.13	3.35	0.02
CSDSP	1.39	0.04	41.85	2.73	3.41	33.63	0.06

Table A.7 Other hydrochemical variables from streams impacted by metal mine drainage

	ph	COND (µS)	T (ºC)	TDS (ppm)	Flow (m3/s)	SS (mg/l)	Width (m)	DO (%)
DUS	6.67	61.64	14.00	41.73	0.73	3.65	0.95	
DDS	6.70	97.40	13.20	65.85	2.69	0.00	3.30	
FUS	6.91	118.10	14.40	79.65	0.53	3.96	0.85	
FDS	6.65	121.20	15.20	81.61	0.86	2.12	1.60	
CYUS	7.33	46.66	15.00	31.54	59.72	0.38	10.58	
CYDS	7.20	63.51	13.70	43.02	21.74	0.00	9.73	
CSUS	6.63	63.42	19.80	42.35	0.58	0.41	0.60	101.00
CSDS	6.92	134.60	15.90	90.35	0.61	0.40	1.35	121.00

Table A.7 Continuation Other hydrochemical variables from streams impacted by metal mine drainage

	ph	COND (µS)	T (ºC)	TDS (ppm)	Flow (m3/s)	SS (mg/l)	Width (m)	DO (%)
DUA	6.88	51.84	6.60	34.99	22.12	0.59	0.95	92.00
DDA	6.96	58.54	7.30	39.47	52.99	2.75	3.30	92.00
FUA	6.50	76.70	8.60	51.50	5.24	0.39	0.85	87.00
FDA	6.50	81.10	9.00	81.10	16.23	0.00	1.60	97.00
CYUA	6.50	37.80	7.60	25.40	458.20	1.60	10.58	95.00
CYDA	6.50	40.80	7.30	27.40	241.69	0.98	9.73	95.00
CSUA	6.31	58.45	7.50	39.37	5.32	5.28	0.60	96.00
CSDA	6.50	89.93	8.20	60.25	41.04	2.59	1.35	96.00
DUW	7.42	59.57	5.40	41.35	15.63	0.38	0.95	104.20
DDW	7.18	71.46	6.10	49.54	65.60	0.74	3.30	101.60
FUW	6.90	74.70	7.90	51.60	9.33	1.84	0.85	95.60
FDW	6.80	78.60	8.40	54.20	13.97	2.16	1.60	102.90
CYUW	7.40	62.10	6.50	43.10	345.90	4.81	10.58	104.30
CYDW	7.20	38.30	6.60	26.57	149.76	0.96	9.73	104.60
CSUW	8.04	70.06	7.30	48.45	8.67	2.08	0.60	104.60
CSDW	8.50	100.90	8.60	69.48	19.22	2.12	1.35	104.20
DUSP	7.40	50.41	11.80	35.24	2.95	1.15	0.95	121.80
DDSP	7.07	71.15	12.40	49.66	26.53	0.38	3.30	118.00
FUSP	6.84	76.72	9.20	54.04	0.74	0.59	0.85	94.50
FDSP	6.73	85.34	10.40	59.95	2.18	0.96	1.60	101.10
CYUSP	7.19	37.93	8.30	26.79	106.56	0.00	10.58	105.00
CYDSP	6.64	46.40	8.20	32.84	55.70	0.00	9.73	120.00
CSUSP	7.11	73.50	13.50	51.09	0.49	0.59	0.60	105.90
CSDSP	7.18	113.60	12.50	19.44	7.54	0.00	1.35	

APPENDIX B. SPECIES LIST

Table B.1 Ephemeroptera and Plecoptera species found in the study streams

Ephemeroptera	Beatidae	*Beatis rhodani*
		Beatis niger
		Beatis vernus
		Beatis muticus
		Centroptilum luteum
		Procloeon bifidum
	Ephemerellidae	*Ephemerella ignita*
	Heptagenidae	*Ecdyonurus venosus*
		Ecdyonurus insignis
		Heptagenia sulphurea
		Rhithrogena semicolorata
	Leptophlebiidae	*Paraleptophlebia cincta*
		Paraleptophlebia werneri
		Paraleptophlebia submarginata
		Habrophlebia fusca
	Ephemeridae	*Ephemera danica*
	Caenidae	*Caenis robusta*
Plecoptera	Leuctridae	*Leuctra moselyi*
		Lecutra inermis
		Lecutra hipopus
		Leuctra nigra
		Leuctra fusca
	Nemouridae	*Numerella picteti*
		Nemoura cambrica
		Nemoura cinerea
		Nemoura erratica
		Nemoura avicularis
		Protonemura praecox
		Protonemura geniculata
		Amphinemura sulcicollis
	Chloroperlidae	*Chloroperla torrentium*
		Chloroperla tripunctata
	Perlolidae	*Isoperla grammatica*
		Perlodes microcephala
		Brachyptera risi

Table B.2 Trichoptera species found in the study streams

Trichoptera	Hydropsychidae	*Hydropsyche siltalai*
		Hydropsyche angustipennis
		Hydropsyche fulvipes
		Hydropsyche conturbernalis
	Goeridae	*Goera pallipes*
		Silo pallipes
	Rhyacophilidae	*Rhyacophila dorsalis*
		Riacophilia obliterata
	Sericostomatidae	*Sericostoma personatum*
	Limnephilidae	*Potamophylax rotundipensis*
		Chaetopteryx villosa
		Micropterna lateralis
		Micropterna sequax
		Glyphotaelius pellucidus
		Apatania muliebris
	Hydroptilidae	*Orsthotridia*
	Phryganeidae	*Agryonia*
	Polycentropodidae	*Plectrocnemia geniculata*
		Plectrocnemia conspersa
	Psychomyiidae	*Lype reducta*
		Lype phaeopa
		Tinodes maculicornis
		Crunoecia irrorata
		Wormaldia occipitalis
	Glossosomatidae	*Glossosoma boltoni*
		Glossosoma conformis
		Glossosoma spp
		Agapepus fuscipes
	Odontoceridae	*Odontocerum albicorne*

Table B.3 Chironomidae species found in the study streams

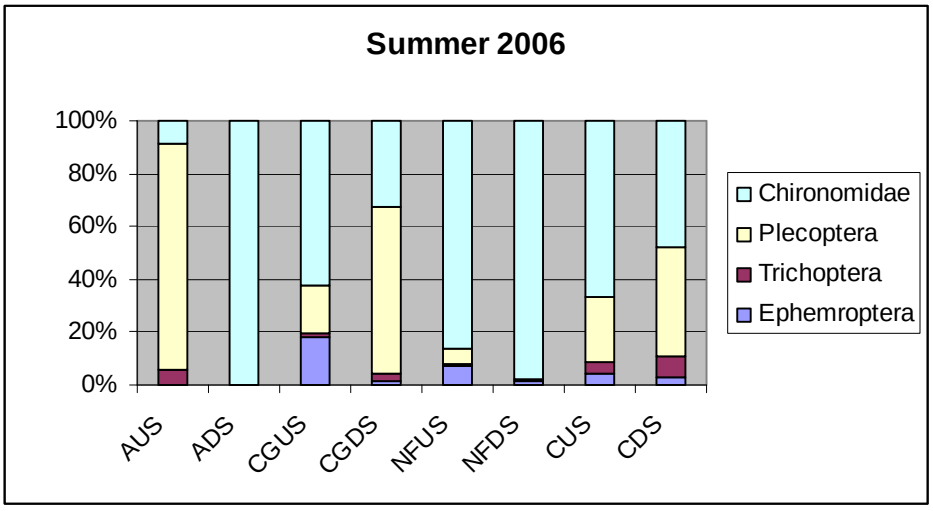
Chironomidae	Orthocladiinae	*Eukiefferiella sp C*
		Eukiefferiella brevicalcar-type
		Eukiefferiella claripennis
		Eukieferiella minor
		Eukiefferiella devonica
		Eukiefferiella gracei
		Eukieferiella clypeata
		Brillia modesta
		Rheicricotopus fuscipes
		Rheicricotopus effusus
		Corynoneura lobata
		Heterotrissocladius marcidus
		Heterotrissocladius grimshawi
		Thienemanniella clavicornis
		Thienemanniella species B
		Chaetocladius piger-type
		Chaetocladius A
		Orthocladius species A
		Paratissocladius exceptus
		Cricotopus annulator
		Cricotopus trianulatus type1
		Cricotopus trianulatus type 2
		Heterotanytarsus
		Symposiocladius
		Einfeldia dissidens
		Heleniella
		Synorthocladius semivirens
		Psectrocladius psilopterns
		Psectrocladius sordidellus
		Paratrichocladius
		Krenosmittia
		Metrocniemus
		Nanocladius rectinervis
		Paratrichocladius skirwithensis
		Paratrichocladius tB
	Diamesinae	*Potthatsia*

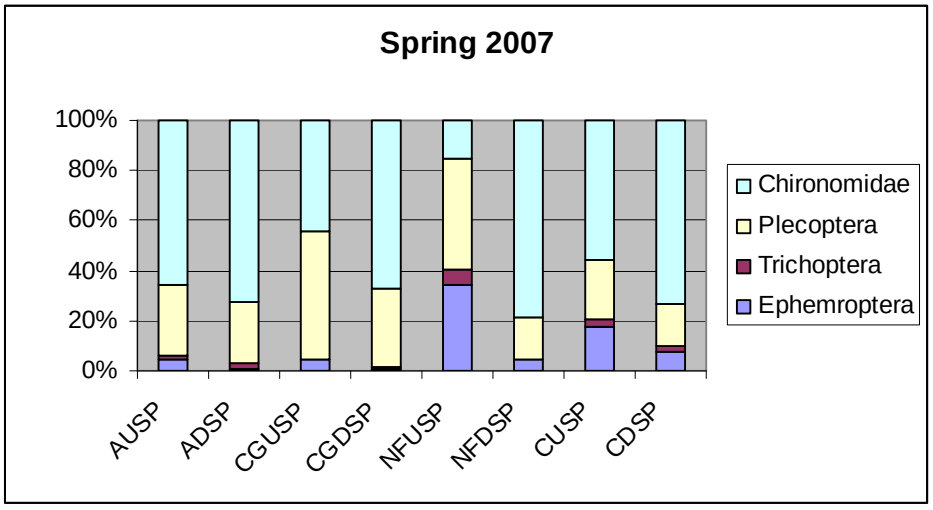
Table B.3 Continuation Chironomidae species found in the study streams

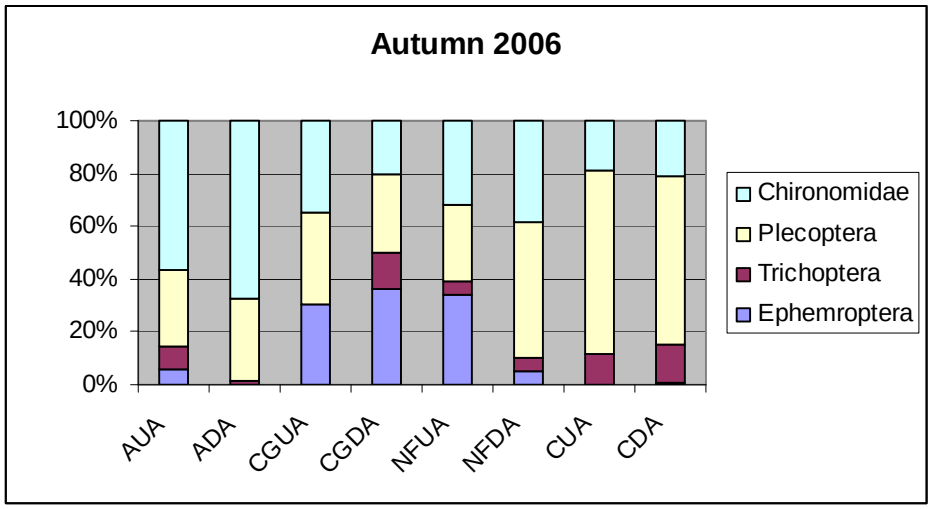
Chironomidae		
	Chironomini	*Paratendipes nudisquama-type*
		Paratendipes albimanus-type
		Polypedilum nubeculosum-type
		Polypedilum sordens-type
		Dicrotendipes nervosus-type
		Dicrotendipes notatus-type
		Phaenospectra flavipens
		Chironomus anthracinus
		Paracladopelma
	Prodiamesinae	*Prodiamesa*
	Tanytarsini	*Tanytarsus mendax-type*
		Tanytarsus nemorosus-type
		Tanytarsus pallidicornis-type
		Tanytarsus chynyesis-type 1
		Rheotanytarsus
		Micropsectra pallidula-type
		Microspectra type A
		Micropsectra radialis-type
		Microspectra contracta-type
		Stempellinella
	Tanypodinae	*Macropelopia*
		Pentaneurini undif
		Procladius

APPENDIX C. EPTC COMPOSITION AT THE STUDY STREAMS

EPTC (Ephemeroptera, Plecoptera, Trichoptera and Chironomidae) composition of streams impacted by coal mine drainage



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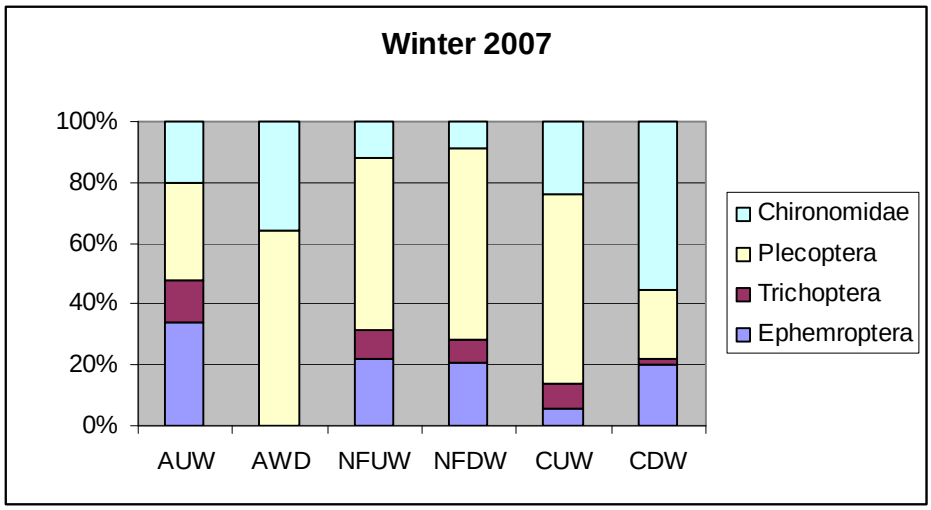
Chironomidae
Plecoptera
Trichoptera
Ephemroptera


Winter 2007
100%
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AUW
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NFUW
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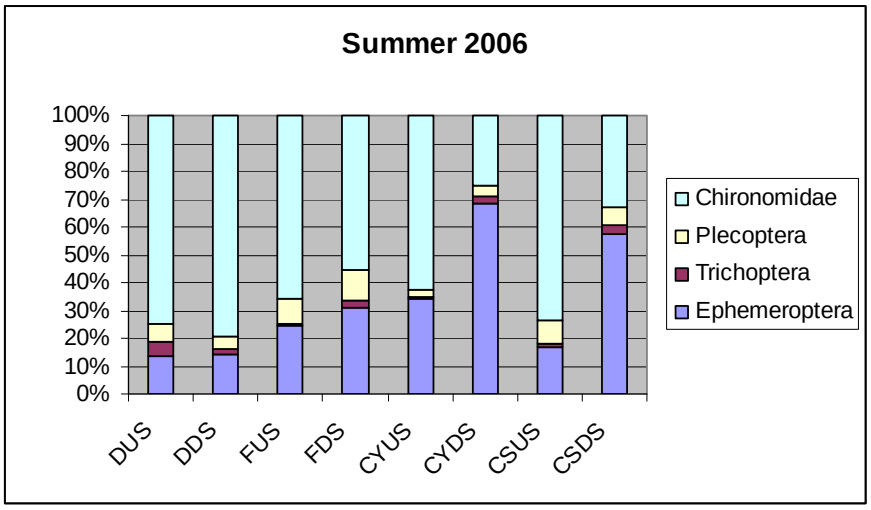
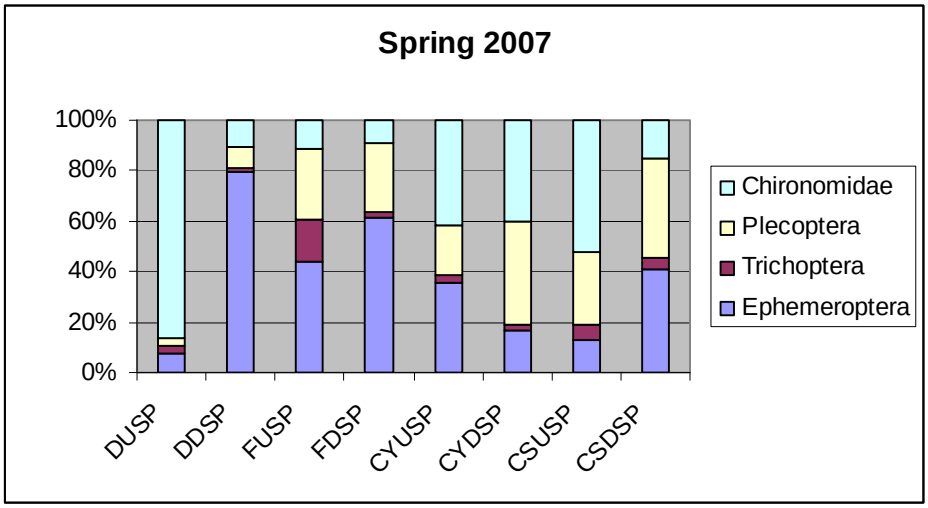
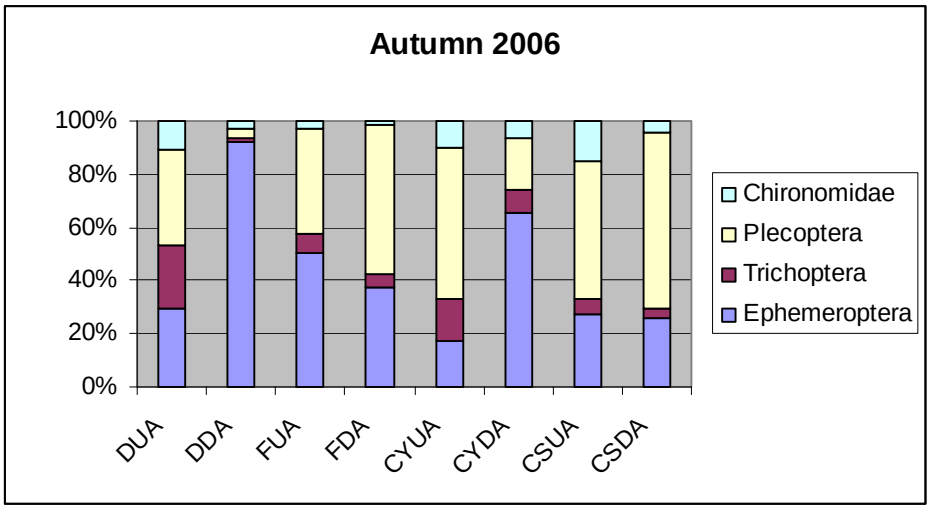
CUW
CDW
Chironomidae
Plecoptera
Trichoptera
Ephemroptera

EPTC composition of streams impacted by metal mine drainage



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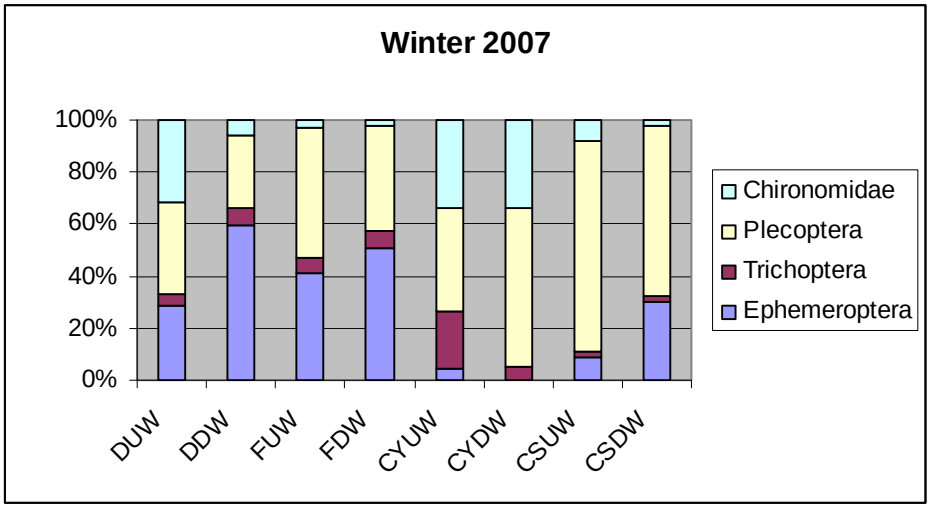
Chironomidae
Plecoptera
Trichoptera
Ephemeroptera


Winter 2007
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FUW
FDW
CYUW
CYDW
CSUW
CSDW
Chironomidae
Plecoptera
Trichoptera
Ephemeroptera

REFERENCE LIST

ADLER, P. R. & SIBRELL, P. L. (2003) Sequestration of phosphorus by acid mine drainage floc. *Journal of Environmental Quality,* 32**,** 1122-1129.

ADMIRAAL, W., BLANCK, H., BUCKERT-DE JONG, M., GUASCH, H., IVORRA, N., LEHMANN, V., NYSTROM, B. A. H., PAULSSON, M. & SABATER, S. (1999) Short-term toxicity of zinc to microbenthic algae and bacteria in a metal polluted stream. *Water Research,* 33**,** 1989-1996.

APHA, AWWA & WPCF (1976) *Standard methods for the examination of water and wastewater*. Washington, American Public Health Association

ARMITAGE, D. (1980) The effects of mine drainage and organic enrichment on benthos in the river Nent system, Northern Pennines. *Hydrobiologia,* 74**,** 119-128.

ARNEKLEIV, J. V. & STORSET, L. (1995) Downstream effects of mine drainage on benthos and fish in a Norwegian river - a comparison of the situation before and after river rehabilitation. *Journal of Geochemical Exploration,* 52**,** 35-43.

BALISTRIERI, L. S. & MURRAY, J. W. (1986) The surface-chemistry of sediments from the Panama basin - the influence of Mn oxides on metal adsorption. *Geochimica Et Cosmochimica Acta,* 50**,** 2235-2243.

BATTAGLIA, M., HOSE, G. C., TURAK, E. & WARDEN, B. (2005) Depauperate macroinvertebrates in a mine affected stream: Clean water may be the key to recovery. *Environmental Pollution,* 138**,** 132-141.

BATTY, L. C. & YOUNGER, P. L. (2002) Critical role of macrophytes in achieving low iron concentrations in mine water treatment wetlands. *Environmental Science & Technology,* 36**,** 3997-4002.

BATTY, L. C. & YOUNGER, P. L. (2004) The use of waste materials in the passive remediation of mine water pollution. *Surveys in Geophysics,* 25**,** 55-67.

BATTY, L., HOOLEY, D. & YOUNGER, P. (2008) Iron and manganese removal in wetland treatment systems: Rates, processes and implications for management. *Science of the Total Environment,* 402**,** 147-147.

BELL, H. L. (1971) Effect of Low pH on survival and emergence of aquatic Insects. *Water Research,* 5**,** 313-319.

BENFIELD, E. F. (1996) Leaf breakdown in stream ecosystems. IN HAUER, F. R. & LAMBERTI, G. A. (Eds.) *Methods in stream ecology.* London, Academic press.

BERMINGHAM, S., MALTBY, L. & COOKE, R. C. (1996a) Effects of a coal mine effluent on aquatic hyphomycetes.1. Field study. *Journal of Applied Ecology,* 33**,** 1311-1321.

BERMINGHAM, S., MALTBY, L. & COOKE, R. C. (1996b) Effects of a coal mine effluent on aquatic hyphomycetes.2. Laboratory toxicity experiments. *Journal of Applied Ecology,* 33**,** 1322-1328.

BERVOETS, L. & BLUST, R. (2000) Effects of pH on cadmium and zinc uptake by the midge larvae *Chironomus riparius*. *Aquatic Toxicology,* 49**,** 145-157.

BERVOETS, L., BLUST, R., DEWIT, M. & VERHEYEN, R. (1997) Relationships between river sediment characteristics and trace metal concentrations in tubificid worms and chironomid larvae. *Environmental Pollution,* 95**,** 345-356.

BERVOETS, L., SOLIS, D., ROMERO, A. M., VAN DAMME, P. A. & OLLEVIER, F. (1998) Trace metal levels in chironomid larvae and sediments from a Bolivian river: Impact of mining activities. *Ecotoxicology and Environmental Safety,* 41**,** 275-283.

BGS (1997) Geology of the country around Llanfair and Rhayader. IN NERC (Ed.).

BICK, D. E. (1974) *The old metal mines of Mid-Wales. Cardiganshire-South of Devil's Bridge,* Gloucester, The Pound House.

BICK, D. E. (1975) *The old metal mines of Mid-Wales. Cardiganshire - The Rheidol to Goginan,* Gloucester, The Pound House.

BICK, D. E. (1976) *The old metal mines of Mid-Wales. Cardiganshire - North of Goginan,* Gloucester, The Pound House.

BICK, D. E. (1977) *The old metal mines of Mid-Wales. West Montgomeryshire,* Gloucester, The Pound House.

BIRD, G. A., ROSENTRETER, M. J. & SCHWARTZ, W. J. (1995) Deformities in the menta of chironomid larvae from the experimental lakes Area, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences,* 52**,** 2290-2295.

BLAKELY, T. J., HARDING, J. S., MCINTOSH, A. R. & WINTERBOURN, M. J. (2006) Barriers to the recovery of aquatic insect communities in urban streams. *Freshwater biology,* 51**,** 1634-1645.

BORCARD, D., LEGENDRE, P. & DRAPEAU, P. (1992) Partialling out the spatial component of ecological variation. *Ecology,* 73**,** 1045-1055.

BOYERO, L. & BOSCH, J. (2004) The effect of riffle-scale environmental variability on macroinvertebrate assemblages in a tropical stream. *Hydrobiologia,* 524**,** 125-132.

BRABEC, K., ZAHRADKOVA, S., NEMEJCOVE, D., PARIL, P., KOKES, J. & JARKOVSKY, J. (2004) Assessment of organic pollution effect considering differences between lotic and lentic habitats. *Hydrobiologia,* 516**,** 331-346.

BRADLEY, D. C. & ORMEROD, S. J. (2002) Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater biology,* 47**,** 161-171.

BROOKS, S. J., LANGDOM, P. G. & HEIRI, O. (2007) *The identification and use of Palaearctic Chironomidae larvae in Palaeoecology,* London, Quaternary Research Association.

BROWN, A. V. & BRUSSOCK, P. P. (1991) Comparisons of benthic invertebrates between riffles and pools. *Hydrobiologia,* 220**,** 99-108.

BROWN, B. E. (1977) Effects of mine drainage on river Hayle, Cornwall .A. Factors affecting concentrations of copper, zinc and iron in water, sediments and dominant invertebrate fauna. *Hydrobiologia,* 52**,** 221-233.

BUSS, D., BAPTISTA, D., NESSIMIAN, J. & EGLER, M. (2004) Substrate specificity, environmental degradation and disturbance structuring macroinvetebrate assemblages in neotropical streams. *Hydrobiologia,* 518**,** 179-188.

BUTLER, B. A., RANVILLE, J. F. & ROSS, P. E. (2008) Observed and modeled seasonal trends in dissolved and particulate Cu, Fe, Mn, and Zn in a mining-impacted stream. *Water Research,* 42**,** 3135-3145.

BUZBY, K. M. & VIADERO, R. C. (2007) Structural and functional aspects of treated mine water and aquaculture effluent streams. *Hydrobiologia,* 583**,** 251-263.

CAINES, L. A., WATT, A. W. & WELLS, D. E. (1985) The uptake and release of some trace-metals by aquatic bryophytes in acidified waters in Scotland. *Environmental Pollution Series B-Chemical and Physical,* 10**,** 1-18.

CAIRNS, J., CROSSMAN, J. S., DICKSON, K. L. & HERRICKS, E. E. (1971) The recovery of damaged streams. *Association of southeastern biologist Bulletin,* 18**,** 79-106.

CAMARGO, J. A., ALONSO, K. & DE LA PUENTE, M. (2005) Eutrophication downstream from small reservoirs in mountain rivers of Central Spain. *Water Research,* 39**,** 3376-3384.

CANOVAS, C. R., HUBBARD, C. G., OLIAS, M., NIETO, J. M., BLACK, S. & COLEMAN, M. L. (2008) Hydrochemical variations and contaminant load in the Rio Tinto (Spain) during flood events. *Journal of Hydrology,* 350**,** 25-40.

CARDINALE, B. J., SRIVASTAVA, D. S., DUFFY, J. E., WRIGHT, J. P., DOWNING, A. L., SANKARAN, M. & JOUSEAU, C. (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature,* 443**,** 989-992.

CARLISLE, D. M. & CLEMENTS, W. H. (2005) Leaf litter breakdown, microbial respiration and shredder production in metal-polluted streams. *Freshwater Biology,* 50**,** 380-390.

CHAPIN, F. S., WALKER, B. H., HOBBS, R. J., HOOPER, D. U., LAWTON, J. H., SALA, O. E. & TILMAN, D. (1997) Biotic control over the functioning of ecosystems. *Science,* 277**,** 500-504.

CHERRY, D. S., CURRIE, R. J., SOUCEK, D. J., LATIMER, H. A. & TRENT, G. C. (2001) An integrative assessment of a watershed impacted by abandoned mined land discharges. *Environmental Pollution,* 111**,** 377-388.

COSTA, S. S. & MELO, A. S. (2008) Beta diversity in stream macroinvertebrate assemblages: among-site and among-microhabitat components. *Hydrobiologia,* 598**,** 131-138.

COURTNEY, L. A. & CLEMENTS, W. H. (1998) Effects of acidic pH on benthic macroinvertebrate communities in stream microcosms. *Hydrobiologia,* 379**,** 135-145.

COURTNEY, L. A. & CLEMENTS, W. H. (2002) Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: an experimental approach. *Freshwater Biology,* 47**,** 1766-1778.

CRANSTON, P. S. (1982) *A key to the larvae of the British Orthocladiinae (Chironomidae),* Ambleside, Freshwater Biological Association.

CROFT, P. (1986) *A key to the major groups of British freshwater invertebrates,* Shrewsbury, FSC Publications.

CUMMINS, K. W. & KLUG, M. J. (1979) Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics,* 10**,** 147-172.

CUMMINS, K. W. (1974) Structure and function of stream ecosystems. *Bioscience,* 24**,** 631-641.

DE BISTHOVEN, L. J., NUYTS, P., GODDEERIS, B. & OLLEVIER, F. (1998) Sublethal parameters in morphologically deformed Chironomus larvae: clues to understanding their bioindicator value. *Freshwater Biology,* 39**,** 179-191.

DE BISTHOVEN, L. J., POSTMA, J. F., PARREN, P., TIMMERMANS, K. R. & OLLEVIER, F. (1997) Relations between heavy metals in aquatic sediments and in Chironomus larvae of Belgian lowland rivers and their morphological deformities. *Canadian Journal of Fisheries and Aquatic Sciences,* 55**,** 688-703.

DENICOLA, D. M. & STAPLETON, M. G. (2002) Impact of acid mine drainage on benthic communities in streams: the relative roles of substratum vs. aqueous effects. *Environmental Pollution,* 119**,** 303-315.

DEUTSH, W. J. (1997) *Groundwater geochemistry: fundamentals and applications to contamination*, Boca Raton, Fla., Lewis Publishers.

DICKMAN, M. & RYGIEL, G. (1996) Chironomid larval deformity frequencies, mortality, and diversity in heavy-metal contaminated sediments of a Canadian riverine wetland. *Environment International,* 22**,** 693-703.

DIGGINS, T. P. & STEWART, K. M. (1993) Deformities of aquatic larval midges (Chironomidae, Diptera) in the sediments of the Buffalo River, New-York. *Journal of Great Lakes Research,* 19**,** 648-659.

DIONEX (2004) Product Manual: OnGuard II Cartridges.

DSA, J. V., JOHNSON, K. S., LOPEZ, D., KANUCKEL, C. & TUMLINSON, J. (2008) Residual toxicity of acid mine drainage-contaminated sediment to stream macroinvertebrates: Relative contribution of acidity vs. metals. *Water Air and Soil Pollution,* 194**,** 185-197.

DUARTE, S., PASCOAL, C., ALVES, A., CORREIA, A. & CASSIO, F. (2008) Copper and zinc mixtures induce shifts in microbial communities and reduce leaf litter decomposition in streams. *Freshwater Biology,* 53**,** 91-101.

EA (2008) Environment Agency. www.environment-agency.gov.uk

EDINGTON, J. M. & HILDREW, A. G. (1995) *Caseless Caddis Larvae of the British Isles,* Ambleside, Freshwater Biological Association.

EDWARDS, P. & POTTER, H. (2007) The Cwm Rheidol metal mines remediation project - Phase 1. IN CIDU, R. & FRAU, F. (Eds.) *Proceedings of the IMWA Symposium 2007: Water in Mining Environments.* Cagliari, Italy.

ELLIOT, J. M. & HUMPESCH, U. H. (1983) Larvae of the British *Ephemeroptera: a key with ecological notes,* Ambleside, Freshwater Biological Association.

EMERY, J. C., GURNELL, A. M., CLIFFORD, N. J., PETTS, G. E., MORRISSEY, I. P. & SOAR, P. J. (2003) Classifying the hydraulic performance of riffle-pool bedforms for habitat assessment and river rehabilitation design. *River Research and Applications,* 19**,** 533-549.

FABUSS, M. A. & FABUSS, B. M. (1974) Water. IN SNELL, F. D. & ETTRE, L. S. (Eds.) *Encyclopaedia of Industrial Chemical Analysis.* New York, John Wiley & Sons.

FARAG, A. M., NIMICK, D. A., KIMBALL, B. A., CHURCH, S. E., D.D., H. & BRUMBAUGH, W. G. (2007) Concentrations of metal in water, sediment, biofilm, benthic macroinvertebrates, and fish in Boulder River watershed, Montana, and the role of colloids in metal uptake. *Archives of environmental contamination and toxicology,* 52**,** 397-409.

FELDMAN, R. S. & CONNOR, E. F. (1992) The relationship between pH and community structure of invertebrates in streams of the Shenandoah-National-Park, Virginia, USA. *Freshwater Biology,* 27**,** 261-276.

FENOGLIO, S., BO, T., AGOSTA, P. & CUCCO, M. (2005) Mass loss and macroinvertebrate colonisation of fish carcasses in riffles and pools of a NW Italian stream. *Hydrobiologia,* 532**,** 111-122

FLEEGER, J. W., CARMAN, K. R. & NISBET, R. M. (2003) Indirect effects of contaminants in aquatic ecosystems. *The Science of the Total Environment,* 317**,** 207-233.

FLEMMING, C. A. & TREVORS, J. T. (1989) Copper toxicity in fresh-water sediment and Aeromonas-Hydrophila cell-suspensions measured using an O2 electrode. *Toxicity Assessment,* 4**,** 473-485.

FLINTROP, C., HOHLMANN, B., JASPER, T., KORTE, C., PODLAHA, O. G., SCHEELE, S. & VEIZER, J. (1996) Anatomy of pollution: Rivers of north Rhine-Westphalia, Germany. *American Journal of Science,* 296**,** 58-98.

FORROW, D. M. & MALTBY, L. (2000) Towards a mechanistic understanding of contaminant-induced changes in detritus processing in streams: Direct and indirect effects on detritivore feeding. *Environmental Toxicology and Chemistry,* 19**,** 2100-2106.

FOX, J. (2002) *Multiple and generalised nonparametric regressions,* Sage University papers series on quantitative applications in the social sciences, 07-131, Thousand Oaks, Sage.

FU, G. M., ALLEN, H. E. & COWAN, C. E. (1991) Adsorption of cadmium and copper by manganese oxide. *Soil Science,* 152**,** 72-81.

FUGE, R., LAIDLAW, I. M. S., PERKINS, W. T. & ROGERS, K. P. (1991) The influence of acidic mine and spoil drainage on water quality in the Mid-Wales area. *Environmental Geochemistry and Health,* 13**,** 70-75.

GANDY, C. J. & YOUNGER, P. L. (2008) Predicting long-term contamination potential of perched groundwater in a mine-waste heap using a random-walk method. *Hydrogeology Journal,* 16**,** 447-459.

GEOPACKS (2002) *Geopacks flowmeters. Instruction manual*.

GERHARDT, A. (1993) Review of impact of heavy metals on stream invertebrates with special emphasis on acid conditions. *Water Air and Soil Pollution,* 66**,** 289-314.

GHANEM, S. A. & MIKKELSEN, D. S. (1988) Sorption of zinc on iron hydrous oxide. *Soil Science,* 146**,** 15-21.

GILLER, P. S. & MALMQVIST, B. (2008) *The biology of streams and rivers,* Oxford, Oxford University Press.

GOETSCH, P. A. & PALMER, C. G. (1997) Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. *Environmental Contamination Toxicology,* 32**,** 32-41

GOUZINIS, A., KOSMIDIS, N., VAYENAS, D. V. & LYBERATOS, G. (1998) Removal of Mn and simultaneous removal of NH3, Fe and Mn from potable water using a trickling filter. *Water Research,* 32**,** 2442-2450.

GRAY, N. F. (1997) Environmental impact and remediation of acid mine drainage: A management problem. *Environmental Geology,* 30**,** 62-71.

GRAY, N. F. (1998) Acid mine drainage composition and the implications for its impact on lotic systems. *Water Research,* 32**,** 2122-2134.

GRESSENS, S.E. (2007) Temporal and spatial responses of Chironomidae (Diptera) and other benthic invertebrates to urban stormwater runoff. *Hydrobiologia,* 575, 173-190.

GRIMALT, J. O., FERRER, M. & MACPHERSON, E. (1999) The mine tailing accident in Aznalcollar. *Science of the Total Environment,* 242**,** 3-11.

GRUNER, D. S., SMITH, J. E., SEABLOOM, E. W., SANDIN, S. A., NGAI, J. T., HILLEBRAND, H., HARPOLE, W. S., ELSER, J. J., CLELAND, E. E., BRACKEN, M. E. S., BORER, E. T. & BOLKER, B. M. (2008) A cross-system synthesis of consumer and nutrient resource control on producer biomass. *Ecology Letters,* 11**,** 740-755.

HALL, R. J., LIKENS, G. E., FIANCE, S. B. & HENDREY, G. R. (1980) Experimental acidification of a stream in the Hubbard Brook experimental forest, New-Hampshire. *Ecology,* 61**,** 976-989.

HARRAD, S., BATTY, L., DIAMOND, M. & ARHONDITSIS, G. (2008) *Student projects in environmental science,* Chichester, John Wiley & Sons Ltd.

HART, D. D. & FINELLI, C. M. (1999) Physical-biological coupling in streams: The pervasive effects of flow on benthic organisms. *Annual Review of Ecology and Systematics,* 30**,** 363-395.

HEINO, J. (2008) Patterns of functional biodiversity and function-environment relationships in lake littoral macroinvertebrates. *Limnology and Oceanography,* 53**,** 1446-1455.

HEINO, J., MYKRA, H., HAMALAINEN, H., AROVIITA, J. & MUOTKA, T. (2007) Responses of taxonomic distinctness and species diversity indices to anthropogenic impacts and natural environmental gradients in stream macroinvertebrates. *Freshwater Biology,* 52**,** 1846-1861.

HERR, C. & GRAY, N. F. (1997) Metal contamination of riverine sediments below the Avoca mine, south east Ireland. *Environmental Geochemistry and Health,* 19**,** 73-82.

HERRICKS, E. E. & CAIRNS, J. (1977) The effects of lime neutralisation of aid mine drainage on stream ecology. *Proc. 32nd Industrial Waste Conference.*

HERRICKS, E. E. & CAIRNS, J. (1972) The recovery of stream macrobenthic communities from the effects of acid mine drainage. *4th Symposium on Coal Mine Drainage.*

HICKEY, C. W. & CLEMENTS, W. H. (1998) Effects of heavy metals on benthic macroinvertebrate communities in New Zealand streams. *Environmental Toxicology and Chemistry,* 17**,** 2338-2346.

HICKEY, C. W. & GOLDING, L. A. (2002) Response of macroinvertebrates to copper and zinc in a stream mesocosm. *Environmental Toxicology and Chemistry,* 21**,** 1854-1863.

HILL, B. H., WILLINGHAM, W. T., PARRISH, L. P. & MCFARLAND, B. H. (2000) Periphyton community responses to elevated metal concentrations in a Rocky Mountain stream. *Hydrobiologia,* 428**,** 161-169.

HIRST, H., JUTTNER, I. & ORMEROD, S. J. (2002) Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. *Freshwater Biology,* 47**,** 1752-1765.

HOFFMANN, A. & RESH, V. H. (2003) Oviposition in three species of limnephiloid caddisflies (Trichoptera): hierarchical influences on site selection. *Freshwater Biology,* 48**,** 1064-1077.

HOWARTH, R. W., BILLEN, G., SWANEY, D., TOWNSEND, A., JAWORSKI, N., LAJTHA, K., DOWNING, J. A., ELMGREN, R., CARACO, N., JORDAN, T., BERENDSE, F., FRENEY, J., KUDEYAROV, V., MURDOCH, P. & ZHU, Z. L. (1996) Regional nitrogen budgets and riverine N&P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. *Biogeochemistry,* 35**,** 75-139.

HUDSON, L. A. & CIBOROWSKI, J. J. H. (1996) Teratogenic and genotoxic responses of larval *Chironomus salinarius* group (Diptera: Chironomidae) to contaminated sediment. *Environmental Toxicology and Chemistry,* 15**,** 1375-1381.

HUDSON-EDWARDS, K. A., MACKLIN, M. G., BREWER, P. A. & DENNIS, I. A. (2008) Assessment of metal mining-contaminated river sediments in England and Wales. Aberystywth, Environment Agency.

HYNES, H. B. N. (1970) Ecology of Stream Insects. *Annual Review of Entomology,* 15**,** 25-42.

HYNES, H. B. N. (1977) *Adults and Nymphs of the British Stoneflies (Plecoptera),* Ambleside, Freshwater Biological Association.

IVORRA, N., HETTELAAR, J., KRAAK, M. H. S., SABATER, S. & ADMIRAAL, W. (2002) Responses of biofilms to combined nutrient and metal exposure. *Environmental Toxicology and Chemistry,* 21**,** 626-632.

JAIN, C. K. & RAM, D. (1997) Adsorption of lead and zinc on bed sediments of the River Kali. *Water Research,* 31**,** 154-162.

JARVIS, A. P. & YOUNGER, P. L. (1997) Dominating chemical factors in mine water induced impoverishment of two streams in the Durham coalfield, UK. *Chemistry and Ecology,* 13**,** 249-270.

JEAN, G. E. & BANCROFT, G. M. (1986) Heavy metal adsorption by sulfide mineral surfaces. *Geochimica Et Cosmochimica Acta,* 50**,** 1455-1463.

JEYASINGHAM, K. & LING, N. (2000) Seasonal influence on head capsule deformities in *Chironomus zealandicus* (Hudson) (Diptera : Chironomidae). *Hydrobiologia,* 427**,** 75-82.

JOHNSTON, D. & ROLLEY, S. (2008) Abandoned mines and the Water Framework Directive in the United Kingdom. *Proceedings of the International mine water association congress.* Prague.

JONES, J. R. E. (1949) An ecological study of the River Rheidol, north Cardiganshire, Wales. *Journal of Animal Ecology,* 18**,** 67-88.

JONSSON, M. & MALMQVIST, B. (2000) Ecosystem process rate increases with animal species richness: evidence from leaf-eating, aquatic insects. *Oikos,* 89**,** 519-523.

JONSSON, M., DANGLES, O., MALMQVIST, B. & GUEROLD, F. (2002) Stimulating species loss following perturbation: assessing the effects on process rates. *Proceedings of the Royal Society B,* 269**,** 1047-1052.

JONSSON, M., MALMQVIST, B. & HOFFSTEN, P. O. (2001) Leaf litter breakdown rates in boreal streams: does shredder species richness matter? *Freshwater Biology,* 46**,** 161-171.

KEEN, R. (1996) The archaeology of industrial Wales. *Industrial Archaeology review,* 18**,** 63-82.

KELLY, M. G. (1999) Chapter 18. Effects of heavy metals on the aquatic biota. IN PLUMLEE, G. S. & LOGSDON, M. J. (Eds.) *The environmental geochemistry of mineral deposits.* Colorado, Society of Economic Geologists.

KENNEDY, A. J., CHERRY, D. S. & ZIPPER, C. E. (2005) Evaluation of ionic contribution to the toxicity of a coal-mine effluent using *Ceriodaphnia dubia*. *Archives of Environment Contamination Toxicology,* 49**,** 155-162.

KIFFNEY, P. M. & CLEMENTS, W. H. (1996) Size-dependent response of macroinvertebrates to metals in experimental streams. *Environmental Toxicology and Chemistry,* 15**,** 1352-1356.

KIM, J. G., KO, K. S., KIM, T. H., LEE, G. H., SONG, Y., CHON, C. M. & LEE, J. S. (2007) Effect of mining and geology on the chemistry of stream water and sediment in a small watershed. *Geosciences Journal,* 11**,** 175-183.

KIMBALL, B. A., CALLENDER, E. & AXTMANN, E. V. (1995) Effects of colloids on metal transport in a river receiving acid-mine drainage, Upper Arkansas River, Colorado, USA. *Applied Geochemistry,* 10**,** 285-306.

KOBAYASHI, S. & KAGAYA, T. (2009) Colonization of leaf patches at topographycally different locations by insect shredders in a small mountain stream. *Limnology,* 10**,** 143-147.

KORYAK, M., SHAPIRO, M. A. & SYKORA, J. L. (1972) Riffle zoobenthos in streams receiving acid mine drainage. *Water Research,* 6**,** 1239-1247.

LADD, S. C., MARCUS, W. A. & CHERRY, S. (1998) Differences in trace metal concentrations among fluvial morphologic units and implications for sampling. *Environmental Geology,* 36**,** 259-270.

LANGMUIR, D. (1997) *Aqueous Environmental Geochemistry*. London, Prentice Hall.

LARSEN, D. & MANN, R. (2005) Origin of high manganese concentrations in coal mine drainage, eastern Tennessee. *Journal of Geochemical Exploration,* 86**,** 143-163.

LEDGER, M. E. & HILDREW, A. G. (1998) Temporal and spatial variation in the epilithic biofilm of an acid stream. *Freshwater Biology,* 40**,** 655-670.

LEDGER, M. E. & HILDREW, A. G. (2000) Resource depression by a trophic generalist in an acid stream. *Oikos,* 90**,** 271-278.

LEDGER, M. E. & HILDREW, A. G. (2001) Growth of an acid-tolerant stonefly on epilithic biofilms from streams of contrasting pH. *Freshwater Biology,* 46**,** 1457-1470.

LEDGER, M. E. & HILDREW, A. G. (2005) The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution,* 137**,** 103-118.

LEDGER, M. E., HARRIS, R. M. L., MILNER, A. M. & ARMITAGE, P. D. (2006) Disturbance, biological legacies and community development in stream mesocosms. *Oecologia,* 148**,** 682-691.

LEE, G., BIGHAM, J. M. & FAURE, G. (2002) Removal of trace metals by coprecipitation with Fe, Al and Mn from natural waters contaminated with acid mine drainage in the Ducktown Mining District, Tennessee. *Applied Geochemistry,* 17**,** PII S0883-2927(01)00125-1.

LEGENDRE, P. & LEGENDRE, L. (1998) *Numerical Ecology,* Amsterdam, Elsvier Science B.V.

LENAT, D. R. (1993) Using mentum deformities of Chironomus larvae to evaluate the effects of toxicity and organic loading in streams. *Journal of the North American Benthological Society,* 12**,** 265-269.

LEPORI, F. & ORMEROD, S. J. (2005) Effects of spring acid episodes on macroinvertebrates revealed by population data and in situ toxicity tests. *Freshwater Biology,* 50**,** 1568-1577.

LEPORI, F., BARBIERI, A. & ORMEROD, S. J. (2003) Effects of episodic acidification on macroinvertebrate assemblages in Swiss Alpine streams. *Freshwater Biology,* 48**,** 1873-1885.

LEPS, J. & SMILAUER, P. (2007) *Multivariate Analysis of Ecological Data Using CANOCO,* Cambridge, Cambridge University Press.

LORION, C. & KENNEDY, B. (2009) Relationship between deforestation, riparian forest buffers and benthic macroinvertebrates in neotropical streams. *Freshwater Biology,* 54**,** 165-180.

LUNDBERG, P., RANTA, E. & KAITALA, V. (2000) Species loss leads to community closure. *Ecology Letters,* 3**,** 465-468.

MACKLIN, M. G., RIDGWAY, J., PASSMORE, D. G. & RUMSBY, B. T. (1994) The use of overbank sediments for geochemical mapping and contamination assessment: results from selected English and Welsh floodplains. *Applied Geochemistry,* 9**,** 689-700.

MAGURRAN, A. E. (1988) *Ecological diversity and its measurements,* London, Croom Helm.

MALMQVIST, B. & HOFFSTEN, P. O. (1999) Influence of drainage from old mine deposits on benthic macroinvertebrate communities in central Swedish streams. *Water Research,* 33**,** 2415-2423.

MANLY, B. F. J. (1997) *Randomization, bootstrap and Monte Carlo methods in biology,* London, Chapman & Hall.

MATTHAEI, C. D., UEHLINGER, U., MEYER, E. I. & FRUTIGER, A. (1996) Recolonization by benthic invertebrates after experimental disturbance in a Swiss prealpine river. *Freshwater Biology,* 35**,** 233-248.

MAURICE, C. G., LOWE, R. L., BURTON, T. M. & STANFORD, R. M. (1987) Biomass and compositional changes in the periphytic community of an artificial stream in response to lowered pH. *Water Air and Soil Pollution,* 33**,** 165-177.

MARQUES, M. J., MARTINEZ-CONDE, E. & ROVIRA, J. V. (2003) Effects of zinc and lead mining on the benthic macroinvertebrates of a fluvial ecosystem. *Water Air and Soil Pollution,* 148**,** 363-388.

MEREGALLI, G., VERMEULEN, A. C. & OLLEVIER, F. (2000) The use of chironomid deformation in an in situ test for sediment toxicity. *Ecotoxicology and Environmental Safety,* 47**,** 231-238.

MERIGOUX, S. & DOLEDEC, S. (2004) Hydraulic requirements of stream communities: a case study on invertebrates. *Freshwater Biology,* 49**,** 600-613.

MERRIT, R. & CUMMINS, K. W. (1996) *An introduction to the aquatic insects of North America,* Kendal, Hunt Publishing Company.

Met Office (2009) www.metoffice.gov.uk

MILNER, A. M. (1994) System recovery. IN CALOW, P. & PETTS, G. E. (Eds.) *The River's Handbook.* Oxford, Blackwell Scientific.

MOORE, L. R. (1954) The South Wales coalfield. IN TRUEMAN, A. (Ed.) *The coalfields of Great Britain.* London, Edward Arnold.

MORGAN, A. J., KILLE, P. & STURZENBAUM, R. (2007) Microevolution and ecotoxicology of metals in invertebrates. *Environmental Science & Technology,* 41**,** 1085-1096.

MOSS, B. (1997) *Ecology of fresh waters. Man and medium,* Oxford, Blackwell Science.

MULLINGER, N. (2004) Review of environmental and ecological impacts of drainage from abandoned metal mines in Wales. Neath, Environment Agency Wales.

NAEM, S. (2001) Autotrophic-heterotrophic interactions and their impacts on biodiversity and ecosystem functioning. IN KINZIG, A. P., W., P. S. & TILMAN, D. (Eds.) *The functional consequences of biodiversity: empirical progress and theoretical extensions.* Princeton, Princeton University Press.

NELSON, S. M. (2000) Leaf pack breakdown and macroinvertebrate colonization: bioassessment tools for a high-altitude regulated system? *Environmental Pollution,* 110**,** 321-329.

NESBITT, I. (2006) The Recovery of the Clydach Brook following treatment of a minewater discharge. Environment Agency.

NIYOGI, D. K., LEWIS, W. M. & MCKNIGHT, D. M. (2001) Litter breakdown in mountain streams affected by mine drainage: Biotic mediation of abiotic controls. *Ecological Applications,* 11**,** 506-516.

NIYOGI, D. K., LEWIS, W. M. & MCKNIGHT, D. M. (2002a) Effects of stress from mine drainage on diversity, biomass, and function of primary producers in mountain streams. *Ecosystems,* 5**,** 554-567.

NIYOGI, D. K., MCKNIGHT, D. M. & LEWIS, W. M. (2002b) Fungal communities and biomass in mountain streams affected by mine drainage. *Archiv Fur Hydrobiologie,* 155**,** 255-271.

NUTTALL, C. A. & YOUNGER, P. L. (2000) Zinc removal from hard, circum-neutral mine waters using a novel closed-bed limestone reactor. *Water Research,* 34**,** 1262-1268.

OLOFSSON, B. & LUNDMARK, A. (2009) Monitoring the impact of de-icing salt on roadside soils with time-lapse resistivity measurements. *Environmental Geology,* 57**,** 217-229.

ORMEROD, S. J., BOOLE, P., MCCAHON, C. P., WEATHERLEY, N. S., PASCOE, D. & EDWARDS, R. W. (1987a) Short-term experimental acidification of a Welsh stream - comparing the biological effects of hydrogen-ions and aluminium. *Freshwater Biology,* 17**,** 341-356.

ORMEROD, S. J., WADE, K. R. & GEE, A. S. (1987) Macro-floral assemblages in upland Welsh streams in relation to acidity, and their importance to invertebrates. *Freshwater Biology,* 18**,** 545-557.

PARKMAN, R. H., CURTIS, C. D., VAUGHAN, D. J. & CHARNOCK, J. M. (1996) Metal fixation and mobilisation in the sediments of the Afon Goch estuary - Dulas bay, Anglesey. *Applied Geochemistry,* 11**,** 203-210.

PARSONS, J. D. (1977) Effects of acid mine wastes on aquatic ecosystems. *Water Air and Soil Pollution,* 7**,** 333-354.

PEETERS, E., DEWITTE, A., KOELMANS, A. A., VAN DER VELDEN, J. A. & DEN BESTEN, P. J. (2001) Evaluation of bioassays versus contaminant concentrations in explaining the macroinvertebrate community structure in the Rhine-Meuse delta, the Netherlands. *Environmental Toxicology and Chemistry,* 20**,** 2883-2891.

PETTIGROVE, V. & HOFFMANN, A. (2003) Impact of urbanizationon heavy metals contamination in urban stream sediments: influence of cathcment geology. *Australasian Journal of Ecotoxicology,* 9**,** 119-128.

PETTIGROVE, V. & HOFFMANN, A. (2005) A field-based microcosm method to assess the effects of polluted urban stream sediments on aquatic macroinvertebrates. *Environmental Toxicology and Chemistry,* 24**,** 170-180.

PINDER, L. C. V. (1983) The larvae of Chironomidae (Diptera) of the Holartic region. Introduction. IN WIEDERHOLM, T. (Ed.) *Chironomidae of the Holartic region. Keys and diagnosis. Part 1. Larvae.* Motala, Entomologica Scandinavica.

RABENI, C. F., DOISY, K. E. & ZWEIG, L. D. (2005) Stream invertebrate community functional responses to deposited sediment. *Aquatic Sciences,* 67**,** 395-402.

RAINBOW, P. S. (2002) Trace metal concentrations in aquatic invertebrates: why and so what? *Environmental Pollution,* 120**,** 497-507.

RESH, V. H. & JACKSON, J. K. (1993) Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. IN ROSENBERG, D. M. & RESH, V. H. (Eds.) *Freshwater biomonitoring and benthic macroinvertebrates.* New York, Chapman and Hall.

RICHARDS, K. (1982) *Rivers. Form and process in alluvial channels,* London, Methuen & Co.

RICHARDSON, J. S. & KIFFNEY, P. M. (2000) Responses of a macroinvertebrate community from a pristine, southern British Columbia, Canada, stream to metals in experimental mesocosms. *Environmental Toxicology and Chemistry,* 19**,** 736-743.

RIJSTENBIL, J. W., DEHAIRS, F., EHRLICH, R. & WIJNHOLDS, J. A. (1998) Effect of the nitrogen status on copper accumulation and pools of metal-binding peptides in the planktonic diatom *Thalassiosira pseudonana*. *Aquatic Toxicology,* 42**,** 187-209.

RIKARD, M. & KUNKLE, S. (1990) Sulfate and conductivity as field indicators for detecting coal-mining pollution. *Environmental Monitoring and Assessment,* 15**,** 49-58.

ROSEMOND, A. D., REICE, S. R., ELWOOD, J. W. & MULHOLLAND, P. J. (1992) The effects of stream acidity on benthic invertebrate communities in the South-Eastern United-States. *Freshwater Biology,* 27**,** 193-209.

RUSE, L. P., HERRMANN, S. J. & SUBLETTE, J. E. (2000) Chironomidae (Diptera) species distribution related to environmental characteristics of the metal-polluted Arkansas River, Colorado. *Western North American Naturalist,* 60**,** 34-56.

SABATER, S., GUASCH, H., RICART, M., ROMANI, A., VIDAL, G., KLUNDER, C. & SCHMITT-JANSEN, M. (2007) Monitoring the effect of chemicals on biological communities. The biofilm as an interface. *Analytical and Bioanalytical Chemistry,* 387**,** 1425-1434.

SAETHER, O. A. (1979) Chironomid communities as water quality indicators. *Holartic Ecology,* 2**,** 65-74.

SANCHEZ, J., VAQUERO, M. C. & LEGORBURU, I. (1994) Metal pollution from old lead-zinc mine works - biota and sediment from Oiartzun-Valle. *Environmental Technology,* 15**,** 1069-1076.

SASAKI, A., ITO, A., AIZAWA, J. & UMITA, T. (2005) Influence of water and sediment quality on benthic biota in an acidified river. *Water Research,* 39**,** 2517-2526.

SCHINDLER, D. W., MILLS, K. H., MALLEY, D. F., FINDLAY, D. L., SHEARER, J. A., DAVIES, I. J., TURNER, M. A., LINSEY, G. A. & CRUIKSHANK, D. R. (1985) Long-term ecosystem stress - the effects of years of experimental acidification on a small lake. *Science,* 228**,** 1395-1401.

SCULLION, J. & EDWARDS, R. W. (1980) Effects of coal industry pollutants on the macroinvertebrate fauna of a small river in the South-Wales coalfield. *Freshwater Biology,* 10**,** 141-162.

SCHULTHEIS, A. S., SANCHEZ, M. & HENDRICKS, A. C. (1997) Structural and functional responses of stream insects to copper pollution. *Hydrobiologia,* 346**,** 85-93.

SEPA (2009) Scottish Environment Protection Agency, www.sepa.org.uk

SERVIA, M. J., COBO, F. & GONZALEZ, M. A. (1998) Deformities in larval *Prodiamesa olivacea* (Meigen, 1818) (Diptera, Chironomidae) and their use as bioindicators of toxic sediment stress. *Hydrobiologia,* 385**,** 153-162.

SERVIA, M. J., COBO, F. & GONZALEZ, M. A. (2004) Multiple-trait analysis of fluctuating asymmetry levels in anthropogenically and naturally stressed sites: A case study using *Chironomus riparius* larvae. *Environmental Monitoring and Assessment,* 90**,** 101-112.

SIDLE, R. C., CHAMBERS, J. C. & AMACHER, M. C. (1991) Fate of heavy-metals in an abandoned lead-zinc tailings Pond. 2. Sediment. *Journal of Environmental Quality,* 20**,** 752-758.

SKINNER, W. D. & ARNOLD, D. E. (1990) Short-term biotic response before and during the treatment of an acid mine drainage with sodium carbonate. *Hydrobiologia,* 199**,** 229-235.

SHANNON, C. E. (2006) The impact of metal mine drainage on the water quality and salmonid populations of the Afon Rheidol, Aberystwyth. *Engineering and Physical Sciences.* Manchester, The University of Manchester

SMITH, K. S. (1999) Metal sorption on mineral surfaces: an overview with examples relating to mineral deposits. IN PLUMLEE, G. S. & LOGSDON, M. J. (Eds.) *The environmental geochemistry of mineral deposits.* Colorado, Society of economic geologists.

SMOLDERS, A. J. P., LOCK, R. A. C., VAN DER VELDE, G., MEDINA HOYOS, J. G. & ROELOFS, J. G. M. (2003) Effects of mining activities on heavy metal concentrations in water, sediment, and macroinvertebrates in different reaches of the Pilcomayo River, South America. *Archives of Environmental Contamination and Toxicology,* 44**,** 314-323.

SOIL SURVEY OF ENGLAND AND WALES (1983) Legend for the 1:250,000 Soil Map of England and Wales, Lawes Agricultural Trust, Harpenden

SPANHOFF, B., BISCHOF, R., BOHME, A., LORENZ, S., NEUMEISTER, K., NOTHLICH, A. & KUSEL, K. (2007) Assessing the impact of effluents from a modern wastewater treatment plant on breakdown of coarse particulate organic matter and benthic macroinvertebrates in a lowland river. *Water Air and Soil Pollution,* 180**,** 119-129.

SRIDHAR, K. R., KRAUSS, G., BARLOCHER, F., RAVIRAJA, N. S., WENNRICH, R., BAUMBACH, R. & KRAUSS, G. J. (2001) Decomposition of alder leaves in two heavy metal-polluted streams in central Germany. *Aquatic Microbial Ecology,* 26**,** 73-80.

STEINMAN, A. D. & LAMBERTI, G. A. (1996) Biomass and pigments of benthic algae. IN HAUER, F. R. & LAMBERTI, G. A. (Eds.) *Methods in stream ecology.* London, Academic press.

STERMAN, N. T. (1988) Spectrophotometric and fluorometric chlorophyll analysis. IN LOBBAN, C. S., CHAPMAN, D. J. & KRENER, B. P. (Eds.) *Experimental phycology. A laboratory manual.* Cambridge, Cambridge University Press.

STEVENS, M. M., WARREN, G. N. & BRAYSHER, B. D. (2003) Oviposition response of *Chironomus tepperi* to nitrogenous compounds and bioextracts in two-choice laboratory tests. *Journal of Chemical Ecology,* 29**,** 911-920.

STILLINGS, L. L., FOSTER, A. L., KOSKI, R. A., MUNK, L. & SHANKS, W. C. (2008) Temporal variation and the effect of rainfall on metals flux from the historic Beatson mine, Prince William Sound, Alaska, USA. *Applied Geochemistry,* 23**,** 255-278.

SWAIN, C. H., BREWER, P. A., MACKLIN, M. G. & SIMKIN, J. (2005) The ecological, geomorphological and geochemical controls on river shingle health and development on the Afon Rheidol and Afon Ystwyth, Ceredigion. Bangor, Countryside Council for Wales.

TER BRAAK, C. J. F. & VERDONSCHOT, P. F. M. (1995) Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences,* 57**,** 255-289.

TOMKIEWICZ, S. M. & DUNSON, W. A. (1977) Aquatic insect diversity and biomass in a stream marginally polluted by acid strip mine drainage. *Water Research,* 11**,** 397-402.

TOWNEND, J. (2005) *Practical statistics for environmental and biological scientists,* Chichester, John Wiley & sons Ltd.

UNDERWOOD, A. J. (1996) Spatial and temporal problems. *River restoration* (eds G. E. Petts and P. Callow). Blackwell Science, Oxford.

UKTAG (2008) Proposals for the EQS for annex VIII Substances. UK Technical Advisory Groups on the WFD.

VAN DAMME, P. A., HAMEL, C., AYALA, A. & BERVOETS, L. (2008) Macroinvertebrate community response to acid mine drainage in rivers of the High Andes (Bolivia). *Environmental Pollution,* 156**,** 1061-1068.

VERB, R. G. & VIS, M. L. (2005) Periphyton assemblages as bioindicators of mine-drainage in unglaciated western allegheny plateau lotic systems. *Water Air and Soil Pollution,* 161**,** 227-265.

VERMEULEN, A. C. (1995) Elaborating chironomid deformities as bioindicators of toxic sediment stress - the potential application of mixture toxicity concepts. *Annales Zoologici Fennici,* 32**,** 265-285.

VILLINSKI, J. E., O'DAY, P. A., CORLEY, T. L. & CONKLIN, M. H. (2001) In situ spectroscopic and solution analyses of the reductive dissolution of MnO2 by Fe(II). *Environmental Science & Technology,* 35**,** 1157-1163.

WADHAM, J. L., HODSON, A. J., TRANTER, M. & DOWDESWELL, J. A. (1998) The hydrochemistry of metlwaters draining a polythermal-based, high Arctic glacier, south Svalbard: I. The ablation season. *Hydrological processes,* 12**,** 1825-1849.

WALLACE, I. D., WALLACE, B. & PHILLIPSON, G. N. (1990) *A key to the case-bearing caddis larvae of Britain and Ireland,* Ambleside, Freshwater Biological Association.

WALLACE, J. B. & WEBSTER, J. R. (1996) The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology,* 41**,** 115-139.

WARWICK, W. F. (1990) Morphological deformities in chironomidae (Diptera) larvae from the Lac St-Louis and Laprairie Basins of the St Lawrence River. *Journal of Great Lakes Research,* 16**,** 185-208.

WARWICK, W. F. (1991) Indexing deformities in ligulae and antennae of Procladius larvae (Diptera, Chironomidae) - Application to contaminant-stressed environments. *Canadian Journal of Fisheries and Aquatic Sciences,* 48**,** 1151-1166.

WARWICK, W. F., FITCHKO, J., MCKEE, P. M., HART, D. R. & BURT, A. J. (1987) The incidence of deformities in *Chironomus Spp* from Port-Hope Harbour, Lake-Ontario. *Journal of Great Lakes Research,* 13**,** 88-92.

WATANABE, N. C., HARADA, S. & KOMAI, Y. (2000) Long-term recovery from mine drainage disturbance of a macroinvertebrate community in the Ichi-kawa River, Japan. *Hydrobiologia,* 429**,** 171-180.

WEI, X. C., VIADERO, R. C. & BHOJAPPA, S. (2008) Phosphorus removal by acid mine drainage sludge from secondary effluents of municipal wastewater treatment plants. *Water Research,* 42**,** 3275-3284.

WERNICK, B. G., COOK, K. E. & SCHREIER, H. (1998) Land use and streamwater nitrate-N dynamics in an urban-rural fringe watershed. *Journal of the American Water Resources Association,* 34**,** 639-650.

WHILES, M. R. & WALLACE, J. B. (1992) 1st-year benthic recovery of a headwater stream following a 3-year insecticide-induced disturbance. *Freshwater Biology,* 28**,** 81-91.

WIEDERHOLM, T. (1983) *Chironomidae of the Holartic region. Keys and diagnoses. Part 1. Larvae,* Entomologia Scandinavica.

WIEDERHOLM, T. (1984) Incidence of deformed chironomid larvae (Diptera, Chironomidae) in Swedish lakes. *Hydrobiologia,* 109**,** 243-249.

WILSON, B. & PYATT, F. B. (2007) Heavy metal dispersion, persistence, and bioaccumulation around an ancient copper mine situated in Anglesey, UK. *Ecotoxicology and Environmental Safety,* 66**,** 224-231.

WILSON, S. (2004) Pemberton minewater discharge feasibility study. The Coal Authority.

WILSON, S. (2005) Outline design report for Smithy Brook Park minewater treatment scheme Wigan. The Coal Authority.

WISEMAN, I. M., EDWARDS, P. J. & GRAHAM, P. R. (2002) Ecological recovery of the River Pelenna (South Wales) following the passive treatment of abandoned mine drainage. *National Meeting of the American Society of Mining and Reclamation.* Lexington, Kentuky, ASMR.

WOLLENBERG, A. L. V. D. (1977) Redundancy analysis. An alternative for canonical correlation analysis. *Psychometrika,* 42**,** 207-219.

YOUNGER, P. L. (2000) Nature and practical implications of heterogeneities in the geochemistry of zinc-rich, alkaline mine waters in an underground Fe-Pb mine in the UK. *Applied Geochemistry,* 15**,** 1383-1397.

YOUNGER, P. L., BANWART, S. A. & HEDIN, R. S. (2002) *Mine water: hydrology, pollution, remediation,* London, Kluwer Academic Publishers.

YUDOVICH, Y. E. & KETRIS, M. P. (2006) Chlorine in coal: A review. *International Journal of Coal Geology,* 67**,** 127-144

ZUUR, A. F., LENO, E. N. & SMITH, G. H. (2007) *Analysing ecological data*, New York, Springer.